

Academics in the boardroom

A FEW years ago a colleague working in a university was approached by an establishment of the Ministry of Defence with an invitation to become a consultant on some matters in which he was an expert. He underwent an extensive security grilling (the establishment had an unenviable record of leaks and was sensitive to recent criticism) and a year later was pronounced fit to be consulted. After the first few days at the site he returned to his university where several weeks later he was called by the head of the section for which he had consulted. "I'm terribly sorry to trouble you", he said, "but can you tell me whether you think your daily rate for consulting should be £4, £7 or £10?"

Is consultancy worth anything at all beyond those few pounds sterling either to industry or to universities at present? The question is touched lightly upon in *The Universities and Applied Research*, the proceedings of a day's symposium of industrialists and university scientists earlier this year, published by the Research and Development Society (47 Belgrave Square, London SW1; £4.50). The meeting was organised to look at the relevance of applied research in universities to social and industrial needs. One of the subjects which obviously came in for some attention was the ability of university staff to be responsive to industry—assuming they wished to—and there seemed to have been general agreement that consultancy was a good thing. Thus Professor G. V. R. Born (University of Cambridge) saw consultancy as "a fascinating assignment . . . a less expensive way of providing industry with information and ideas [than taking similar persons on full-time]". And Professor K. Hoselitz (Mullard)—"university consultants (were) a useful way of forming a bridge". And Dr A. Spinks (ICI) "strongly approved of consultants". Can one therefore be satisfied that the consultancy system is an adequate means of broadening the understandings between industry and university, or is there more that could be done in the creation of formal machinery for the movement of highly intelligent people?

Despite the good intentions expressed above, there are many who regard consultancy cynically. On the university side there are those who give very poor value for money, who refuse to see industry in any broader perspective than that of the immediate problem presented to them or who dump the work on their students as unpaid sub-consultants. There are those who arrange with colleagues to provide mutually contradictory advice so that they can profit by regular recapitulation of the same opinion to a confused managing director.

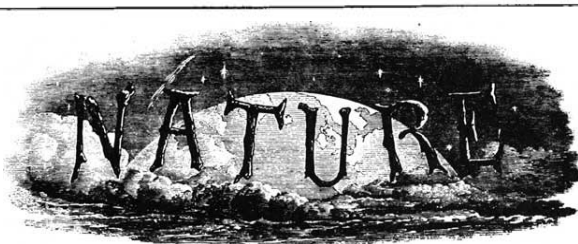
On the industrial side there are many who employ consultants simply because it is a prestigious thing to do. Others do not use consultants for ideas or criticism but to provide an academic rubber stamp for their activities and a reassurance that they are not making asses of themselves.

Obviously the value of a consultancy depends entirely on the calibre of the two parties to it in each case, but there seems to be a need in many instances to go beyond the rather flimsy structure that the arrangement has at present. One of the biggest difficulties of an *ad hoc* scheme is that the consultant is insufficiently tied to the organisation for which he consults. He is not brought far enough into the corporate decision making nor the day-to-day running to feel deeply identified with the need for the company to be profitable. He is also likely to be fed problems which the company believes he might be able to solve—and which the company may have gone to some trouble to concoct for him—rather than be invited to roam around and pick up the problem that seems to him most important.

Many of those in applied research and a few of those in pure research in universities have had some industrial experience, but the number has been declining in recent years. Much of this experience, however, is at the junior scientific and technical level. What is now needed is more opportunities for the process of consultancy to move towards one in which it would be natural for senior university staff to consider a spell of up to several years in industry at the boardroom level. There is no doubt that many academics would find the industrial challenge stimulating and many industries would welcome new blood at the executive level. In addition the still rather distant relationships between industry and university in Britain could not but be helped by this.

The biggest problems are those man-made difficulties of tenure and pensions. It is ridiculous the extent to which careers become controlled by these constraints. There is hope that the quality of some pension schemes and their interchangeability will improve in the next year or two. The problem of university appointments remains and is not easily solved. It needs some imaginative thinking.

A hundred years ago



THE *Journal of the Society of Arts* states that M. Mège Mouriès, after analysing butter, has succeeded in making it synthetically. This imitation butter, recognised by the Conseil d'Hygiène as indistinguishable from real butter, is finding its way into the Paris markets at half the present price of real butter.

From *Nature*, 11, 76, November 26, 1874.



For those in peril: 2

Charlie Clutterbuck, Alan Dalton and Andy Solandt, of the British Society for Social Responsibility in Science, consider what action needs to be taken to identify and regulate industrial health hazards.

OVER the past two decades the petrochemical industry has expanded more rapidly than any other. It is estimated that thousands of new chemicals are introduced into the production processes of the industry each year. One such chemical is vinylchloride monomer (VCM), which is used in the production of one of the world's most important plastics—PVC (polyvinylchloride). Unlike most of the new chemicals introduced into the work environment, VCM has been fairly well studied and was thought to be non-toxic. From being a 'harmless' compound twelve months ago, VCM is now, however, being described as "the occupational hazard of the century" (William Lloyd, United States National Institute for Occupational Health and Safety.) What then might be the dangers of those countless other chemicals that have never been tested as health hazards?

Dr Epstein of Case Western Reserve University Medical School, a specialist on industrial carcinogens, asserts that "In the absence of pre-testing, the worker himself or herself, is unwittingly used as an involuntary test subject, to whom data are not generally available, if indeed they are ever collected and analysed." (Regulatory Aspects of Occupational Carcinogens presented to an International Chemical Federation Conference in Geneva in October 1974.) As a result of this post hoc procedure the health and livelihood of people on the shop floor and in the monitoring labs of industry are in constant jeopardy.

What can the concerned scientist do to help improve this disgraceful situation? One possible answer was suggested by Peter J. Smith in *Nature* (October 18). He proposed that scientists should admit moral responsibility for taking a clear lead in seeing that the ill effects of science and technology are eliminated or at least mitigated." He then went on to suggest that the ways in which scientists could take this 'clear lead' was to do thorough research on health hazards and to ensure that the results of this research be made public. He realised that this work had to be supported by vigorous campaigning to acquire the necessary finances. He also recognised some of the difficulties in

obtaining statistics on health hazards. "Of course, employers frequently attempt to justify their refusal to disclose vital information on the grounds of commercial secrecy—a ploy which is sometimes legitimate, sometimes not."

Unfortunately, however, he was not at all clear on where this information should go and on how it could be presented in order to achieve maximum impact. Clearly the usual procedure of merely publishing in academic journals would accomplish little. Perhaps, as Smith implies in his article, the information should go to a body of elite scientists which would have "an immense potential for influencing public and government opinion." And is it merely opinion that we want to change?

The British Society for Social Responsibility in Science (BSSRS) has been actively combatting some of the hazards of work over the past year. Although this by no means makes us 'experts' in occupational health, it has furnished us with enough experience to enable us to offer a fairly concrete and systematic alternative to Smith's proposals. Before we describe this alternative we describe a case history which is a typical example of the handling of health hazards in industry.

In 1961, a medical officer at a Dow Chemical plant in the United States, discovered that VCM was the probable cause of serious liver damage in a number of men in the plant. On the basis of this, Dow drastically reduced its TLV (threshold limit value or maximum 'safe' time-weighted average) for VCM to 50 p.p.m. This action was made public. But none of the other PVC manufacturers followed Dow's lead. We had to wait until one man, Earl Parkes, had taken B. F. Goodrich of Kentucky to court *twice* to obtain compensation for his liver damage before the knowledge of the dangers of VCM became widely publicised. (Earl Parkes and two other men at B. F. Goodrich subsequently died of the liver cancer angiosarcoma.) So far, one death due to angiosarcoma has been confirmed in Britain. Recently VCM has been associated with lung cancer, painful swelling of the joints of the hands and feet and degeneration of the central nervous system.

Now that the dangers of VCM are widely known, what is being done to protect people from exposure to it? In both the United Kingdom and the United States, industry has adopted emergency standards of 50 p.p.m., with a time-weighted average of 25 p.p.m. The Department of Employment has

set up a special committee to determine a code of practice for VCM; it is important that it reports soon. It has been deliberating for 6 months already and apparently it has not as yet even discussed the problem of a TLV.

Perhaps it will adopt the recent OSHA (Occupational Safety and Health Act in the United States) standard of 1 p.p.m. While this reduction is a welcome improvement however, it must be recognised that the TLV is only a small part of the problem. For even though a relatively low standard has now been set for VCM the OSHA has completely ignored a special committee's report on ways of enforcing this standard. Dr Epstein has made this comment on OSHA's recommendation: "However, the major key provisions of the Committee, including those endorsed by corporate scientists on the Committee, were disregarded by OSHA, presumably under strong industrial pressure." These included recommendations of the committee to ensure the effective implementation of the carcinogen standards by instituting sensitive environmental monitoring systems and a permit system. (Regulatory Aspects of Occupational Carcinogens presented to the ICF Conference, Geneva, 1974.) We can only hope that the new United Kingdom Commission on Health and Safety at Work will prove to be more stringent and independent in its approach to the problem.

It is too early for BSSRS to define clearly 'workers' science'. Even so, there are a number of general guidelines which we can propose at this time based on our own work on VCM hazards at a BP plant in Port Talbot. These guidelines apply to any 'expert', whether technical, medical or scientific, working in the occupational health field. They also apply to those technical people working in industry who feel that there is a clear and immediate need for action on occupational health in their own industries.

- Try to work primarily with the men and women who are actually exposed to the health hazard. This does not mean that one should ignore other groups interested in occupational health. Clearly, both scientific and shop floor workers must seek the help and co-operation of management and government bodies interested in these problems. Management should be consulted and valuable information exchanged. The Factory Inspectorate and/or the Public Health Office should be approached. With the new powers invested in it by the Health and Safety at Work Act, the Factory Inspectorate

may prove to be a valuable partner for any groups seriously working to reduce health hazards in industry.

Liaison with the Trades Union involved is of paramount importance. For it is they who have experience of negotiating with management and they who have at their disposal, the services of the independent Centenary Institute of Occupational Health which specialises in the chemical analysis and medical evaluation of questionable materials.

- Remember that your main reason for being on the 'shop' floor is to exchange information and experience. The people on the shop floor can give you first hand knowledge of the processes they work with and the hazards that these processes involve. They can tell you how their plant/office actually works, not just how it is supposed to work. For your part you can help these men and women to understand the technical/medical explanation of the hazard and how to monitor and keep

the necessary records of the hazard.

- Unfortunately, even the introduction of reasonable health conditions is an issue which often involves conflicts with employers. Recently 5,000 men and women at the Shell/Chevron plant in California had to strike for 5 months just to have seven medical provisions written into their contract. This was done in the light of the fact that all the other major petrochemical companies in the United States had already agreed to medical provisions being written into their contracts. The scientist must be prepared to stand up in defence of working people. This may mean pressing their case in joint management/union committees or even standing up in court to put the facts as they see them. Two men in the United States, Dr Selikoff of Mount Sinai Hospital in New York and Dr Epstein, have actually done this.

- Be prepared to use the press and television to publicise your case. The BSSRS helped World in Action to do

the first exposé of the VCM problem in this country. Later we devoted 15 minutes of an Open Door programme (BBC2) to a discussion of the VCM issue.

- In cooperation with other scientific, production line, and office workers, press for the implementation by the government, management and trade unions of systematic pre-testing of industrial processes and materials for possible health hazards.

Clearly it is not enough for individual scientists to become involved in isolated local health hazard issues. There must be a coordinated body for scientists participating in the field of occupational health. This is precisely the role that the BSSRS has begun to play and hopes to develop systematically and comprehensively in the future. We cannot afford to finance teams of investigation or the much needed thorough long term research in this area. We hope, however, to be able to serve as a vital catalysing agent.

international news

MINISTERS from 24 countries, including Britain's Denis Howell, met in Paris last week at the first meeting of Environment Ministers convened by the Organisation for Economic Cooperation and Development (OECD). The ministers met to discuss several recommendations put forward by the environment group of the OECD on the formulation of rational and coherent environment policies throughout the OECD countries.

One of the concepts discussed at the recent meeting was in fact accepted in outline in 1972, namely the 'Polluter Pays Principle', which in effect means limiting state aid for pollution control to industry while making it conform to certain standards. This will then mean that goods made by polluting industry will be more expensive, as they have to pay for their own pollution control. The consumer, it is argued, will therefore prefer the cheaper goods made by non-polluting industry. Some countries have already incorporated the principle in their legislation, although, exceptionally, state aid may be allowed.

The ministers also discussed the OECD's proposed code of conduct for dealing with the problem of trans-frontier pollution. One of the main points of the code is that pollution exported to other countries must not exceed the levels permitted within the polluting country.

The most widely discussed instance

OECD urges environment policies

by Eleanor Lawrence

of this type of pollution, as far as Britain is concerned, is the sulphur dioxide from Britain and from continental Europe which ends up over Scandinavia as acid rain, with dire environmental consequences. The OECD is at present in the middle of an international survey of air pollution—the first such survey to use completely standardised monitoring methods—which should clarify this situation. Commenting after the meeting, Mr Howell said that Britain might have to reconsider her power-generating policy if the results of the survey showed that sulphur dioxide emissions from power stations were the 'culprit'.

The problem of trans-frontier pollution also raises the question of whether people affected have the same rights in the polluting countries' courts as would citizens of those countries in the same situation. But unfortunately, even the citizens of some countries find it extremely difficult to gain standing on matters of pollution in the courts, so that this might prove to be an empty privilege.

Complications and inequalities could also arise because of the fundamental difference between the judicial structures in the various countries—Europe by and large follows Roman law whereas in Britain there is the added complication of being able to bring prosecutions under common law, which operates on a precedent system rather than on the basis of published statutes.

There is also the problem of who exactly is liable to prosecution. If treaties for lower pollution had actually been signed between polluted and polluting countries, the defendant might well be the Government in the person of the Secretary of State for the Environment. Other problems would, of course, be the usual ones which bedevil pollution legislation generally, such as collecting sufficient data to make a case which will be accepted in the courts in the first place.

At present, if an article manufactured in Britain is sent abroad and proves to be harmful, it may well be the distributors in that country who are prosecuted and not the manufacturing company. This has happened in Australia in the case of thalidomide, marketed there by a subsidiary of Distillers Company Ltd.

The effects of pollution will be far less easy to define in practice than even the effects of a manufactured article, and there is the prospect of a real field day for the lawyers. □

"NOTHING has happened to climate in the past few years that is not the sort of thing that has happened already in the past few centuries". These words from Dr J. S. Sawyer (pictured right) of the UK Meteorological Office, sum up the 'establishment' view of the present concern about climatic doom (see the review on page 335). Serious droughts and floods are part of the climatic pattern, and there seems to be nothing unusual about the present fluctuations. A crash programme of research, says Sawyer, is not the answer to the problems posed by such floods and droughts; there is a need for more people to be active in climatic research, but there is also a need for time for them to grasp the problem as a whole.

Sawyer is also dubious about the value of frequent claims that man's activities are affecting the atmosphere. He likens the situation to a game in which people vie to think up new possible manmade effects, and points out that this is rather futile since there are nowhere near enough people to follow up all the suggestions with detailed studies. He accepts at least one aspect of man's effect on the atmosphere—CO₂ levels are going up as fossil fuel is burnt. With exponential growth, that will produce a rise in temperature by a "just appreciable fraction of a degree Celsius" by the end of this century. And since exponential growth will have



Round Britain

used up all the fossil fuel by then, further extrapolation is fruitless.

• Devotees of the Oxbridge scene will know of the enormous significance attached to keys. College keys are carefully accounted for in annual medieval ceremonies and woe betide those who cannot deliver at the right time. It was with some alarm, then, that we read in *New Horizons*, a Canadian journal of 'frontier' research, of the fate of two such keys belonging to Dr A. R. G. Owen, now editor of that journal and at one time Fellow of Trinity College Cambridge and Lecturer in the Department of Genetics.

On March 8, 1974, Mr U. Geller, who needs no introduction, demonstrated his metal-bending powers on Channel 79, Toronto. The two keys he selected from a large collection were the key to the Genetics Department and a Fellow's key to Trinity, number 13 "registered as issued to me personally". The genetics key was indubitably bent through 15°, though by what process we decline to speculate. Trinity's key remained unbent. Neutral observers will marvel not at Mr Geller's powers but at Dr Owen's ability to evade the Cambridge key audit for so many years.

• In view of the recent accident in Bantry Bay on the west coast of Eire, the development of the Vikoma system for the recovery of spilt oil by BP will be hailed as a guiding light for the petroleum industry. This system incorporates an inflatable boom for containing oil (mainly crude) and a skimmer for recovering it from the surface of the sea.

Primarily designed for BP's own spills, the system has now become commercially available. A towing vessel costs £24,000, and the recovery system £22,000. Although under development since 1967 and already in widespread use, the system has assumed importance as a result of future North Sea oil production.

Arms and the common man

from Wendy Barnaby

A RECENT meeting in Lucerne left a distinct impression that if everything were left to experts—no matter how well intentioned—laboratories would be thriving while the lot of the common man would steadily deteriorate. The meeting, one of a series of conferences being held to update the 1949 Geneva Conventions, was called by the International Committee of the Red Cross to consider the prohibition or restriction of conventional weapons "that may cause unnecessary suffering or have indiscriminate effects". It was hoped that the discussions would pave the way for recommendations to the politicians, whose next conference will be from February to April 1975. Progress was, however, very slow.

Take the case of napalm. The meeting spent some time discussing whether it is an 'all-or-nothing' weapon: that is, whether a person escapes it altogether or is killed by no matter how small an amount he encounters. Statistics of deaths of American soldiers in Vietnam after napalm had accidentally been

dropped on them were quoted to prove that it is not such a weapon. Only four out of 53 men died. But the effectiveness of napalm against personnel varies greatly with the training, experience and equipment of the victims, and with the standard of medical treatment and the rapidity with which it is given. It is therefore spurious to use this figure as a basis for calculating the effects of drops on civilians. Soldiers are trained, equipped and often experienced—and these particular ones received all possible modern medical care 10–20 minutes after the accident. Small comfort for the peasants.

This all-or-nothing sort of argument only confuses the issue by inviting conflicting evidence and adding to the general confusion of the debate. After all, everyone agreed that severe burn wounds are probably the worst possible type of wound. Surely that recognition is more important than a precise determination of how much of the worst possible type of wound is necessary to kill. If military action is aimed at incapacitating the enemy, it is obviously inhumane to achieve this through severe burning when other, less traumatic means are available.

Many argued that the accuracy with which napalm can be delivered makes

its use more discriminate and therefore more humane than less accurate, high explosive and fragmentation weapons. A typical 100-gallon container scatters napalm over a reasonably clearly defined area of about one-quarter of a hectare, whereas artillery of typical accuracy has a circular error probable (the radius of the circle whose centre is the target within which half of the projectiles aimed at the target fall) of 10–20 metres and a range error probable of 30–60 metres. On this basis, napalm was said to have great military value in close air support operations, where it is desired to destroy specific targets near friendly troops. This argument would be all very well if we could be certain that napalm would only be used against structural targets and never against people. Unfortunately such certainty is impossible in any war. The military attractiveness of its accuracy cannot compensate for the risks taken with human suffering.

Similarly, the fact that the experts disagreed on the seriousness of wounds caused by high velocity bullets should not be allowed to paralyse efforts to restrict their use. The problem is urgent because European small-arms manufacturers have developed 5.56 mm guns capable of firing such bullets, which

they are hoping to sell to NATO countries to replace NATO's present 7.62 mm weapons. (In fact it was rumoured at the meeting that Denmark was waiting to hear the outcome of the discussions there before deciding whether to buy just such a new rifle). But again, the evidence was conflicting.

Dr B. Rybeck, the most senior medical officer in the Swedish navy, has carried out experiments on anaesthetised pigs in which he found that high velocity bullets could in general be distinguished from low velocity ones on inspection of the wounds caused by each. In other experiments in Sweden, anaesthetised dogs were shot with metal spheres in order to dissociate the usual tumbling effect of high velocity bullets from their other wounding effects. Blood from a dog which had been shot with a sphere was transfused into a second dog. When the impact velocity of the sphere was 1,000 metres per second the recipient dog suffered severe changes of regional blood flow after the transfusion—an effect not observed when the impact velocity of the sphere was 500 metres per second. Lieutenant Colonel Dr Robert Scott, of the Royal Army Medical Corps, has shot small calibre, high velocity bullets into blocks of gelatin and reported that these did in general produce a greater effect than 7.62 mm bullets, but only at short ranges. If the flight of the bullet into the gelatin was unimpeded, those of high velocity and low calibre deposited more of their kinetic energy in the gelatin than those of larger calibre and lower velocity; however, this was reversed if the bullets passed through a thin steel plate (to simulate the body of a truck, for example, under battle conditions) before entering the gelatin.

The politics of prohibiting the use of a shiny new gun, just when various countries are considering adding it to their armouries, are formidable. And yet there are hopeful signs. In the discussion on napalm, for example, the experts explicitly recognised that the politicians will have to take public opinion into account in determining whether it will be banned. It was even argued that the pressure of public conscience provided grounds for making the use of napalm illegal. So the common man does have a part to play. And he may well argue that he is more interested in establishing the principle of restriction of the use of various weapons, even if some doubtful cases do escape the net, than in allowing the discussions to bog down in technicalities while more countries acquire more harmful arms. In the case of the rifles, even the experts agreed that, in general, wounds caused by low velocity weapons such as pistols, carbines or

submachine guns are much less severe than those caused by rifles. If, on the basis of understandings as common as this, the public were to put enough pressure on the politicians, the experts could be forced to come up with some sort of restricting arrangement. But if the experts are left to devise categorisations of inhumane weapons to present to the politicians as a basis for action, the issues will simply be referred to more laboratories for more experiments the results of which will cause more disagreement. And in the meantime, as inhumane weapons spread to more countries, the danger to the common man will only increase. □

Van Wyk de Vries commission

from Graham Baker, Johannesburg.

SOUTH African universities for the most part enjoy considerable freedom of action in conducting their affairs, subject to the restriction of the country's race laws and in spite of some profound differences of opinion over political legislation between the government and the English-medium institutions in particular. The present undergraduate population of over 80,000 is growing at almost 10% a year and the universities are well aware of their obligation to make up for South Africa's acute shortage of skilled manpower, especially in the sciences and engineering. Furthermore, the government has of late become especially sensitive to the possibility that some universities might have become bases for political activity of which it disapproves.

It is therefore understandable that the academic community in South Africa has been eagerly awaiting the findings of a government-appointed commission of enquiry into white universities, which was tabled in Parliament on October 30. Under the chairmanship of Mr Justice J. van Wyk de Vries, the commission was set up in 1968, principally to consider the financing of universities but also to take a look at "student relations". The recommendations with regard to financing have been openly welcomed as giving the necessary boost for needed expansion. Thus, the commission proposes that up to 85% of running costs should be subsidised by the state and that existing and future capital debts should also be borne by government grants. In return, universities should pay an annual levy of R50 per student to the government. It has been noted that particular advantages from this will accrue to the smaller, more recently established institutions, which tend to be Afrikaans-medium campuses. It also means that universities

can only develop to an extent permitted by the amount of financial assistance provided by the state. Even so, these recommendations, if accepted by the government, are unlikely to come into effect before 1976 at the earliest.

The main objections to the report have been voiced in respect of its views on the non-financial aspects of university life, in particular the extent to which universities may be permitted to engage in political activity. The majority view of the commission is that if a university is politically active in a way the government sees as being 'irregular' and therefore outside its proper function, it will forfeit financial support from the state. Some vice-chancellors see in this a threat that universities must toe the ideological line of the government in power or suffer crippling consequences. They take this as interference with autonomy of the universities and, more bluntly, they see it as an attack on the English-medium universities in South Africa. The Afrikaans-medium institutions, in contrast, see an alignment with government policy as part of their social and political obligation.

Professor G. R. Bozzoli, Principal of the University of the Witwatersrand and a member of the commission, has spoken out strongly against this aspect of the report, as well as another, the 'finding' that South African universities were founded on a social order based on the principal of multinational separate development. As Professor Bozzoli points out, apartheid was only forced on the universities as a result of the Universities Act of 1959.

In a similar light, the commission rejects the view that universities should be free to make appointments without regard to race, colour or creed, and sees no merit in student bodies having representation on university councils and senates.

It happens that the Van Wyk de Vries Commission tabled its report only a few days before the Prime Minister, Mr Vorster, declared in a report-back meeting to his Nigel constituency that he needs just six months to bring about major changes in South Africa's social order, presumably a reference to legislation on race relations. The same message was also spelled out last week by some of Mr Vorster's colleagues at a congress of the Nationalist Party, doubtless a direct consequence of recent events at the United Nations and in neighbouring African states. Whether any moderation of government policy on apartheid will take the heat off the universities and permit any number of black students to be admitted once again to the white institutions, as some vice-chancellors would wish, remains to be seen. □

THE rise of the human rights movement in academic circles in the Soviet Union has led, over the past few years, to an extremely disturbing reaction from the authorities—the incarceration of dissidents in mental institutions. The case of Zhores Medvedev in 1970 is perhaps the best known, but Medvedev's rapid release within a month was an exception, and the average dissident, caught in the penal psychiatric system of the Soviet Union and unable to reap the benefits of a pressure campaign such as that launched by the world scientific community in Medvedev's support, may well expect a considerable stay in an entirely inappropriate establishment.

One such long term internee, Viktor Fainberg, who was released in November 1973 and, after a brief reconfinement in April-May 1974, was finally allowed to emigrate to Israel, visited London recently. He was able to provide considerable background material concerning this abuse of psychiatry. The picture he gives is a horrifying one—of institutions in which the 'doctors' are officers of the MVD, wearing uniforms and bearing military ranks and titles; where convicted criminals serve out their terms in the capacity of male nurses, frequently terrorising the patients and robbing their food parcels; where the treatment facilities for somatic illnesses are scanty (Mr Fainberg himself, who developed thyrotoxicosis during his internment, saw an endocrinologist only twice a year); and where the death rate from the occasional routine operation such as appendectomy is abnormally high because of inadequate post-operative care. Far more alarming in its implications, however, is the picture of false diagnosis and the punitive use of drugs—a picture already publicised in 1973 by Academician Andrei Sakharov, but which emerges in far greater detail from the descriptions of Viktor Fainberg, who has actually been a candidate for such 'treatment'.

The route by which a prospective patient reaches such an institution may vary. Zhores Medvedev was seized without warning. The chemist Anatolii Chinov, caught trying to cross the Soviet-Czechoslovak border in December 1968, himself put forward a plea of insanity, on the advice of his cousin, a psychiatrist, who erroneously thought that this offered him the best chance of rapid release. (This was at the beginning of the new policy). Mr Fainberg himself, who was a member of a small human rights group in Leningrad which was in contact with the better known Moscow group of Sakharov, was sent for a psychiatric report after one warning. In his case, the offence was

that of contempt of court—refusal to testify against five members of his group in whose possession incriminating (dissident) literature was found.

The criteria of selection for psychiatric as opposed to standard legal measures seem somewhat arbitrary. Of the five members of the Leningrad group in question, three (including the chemist Lev Kvachevskii) received terms in labour camps and two were sent to penal psychiatric institutions. Asked if he could suggest any possible basis for the division, Mr Fainberg

Soviet abuse of psychiatry

from Vera Rich, London

said that psychiatric procedures seem to be particularly favoured if the offence in question carries a fairly mild maximum sentence under the criminal code (six months of obligatory work, that is, forfeiture of a percentage of salary in his own case), whereas a 'patient' committed for compulsory psychiatric treatment may be held for an indefinite length of time. It would seem, too, that any background of mild psychological illness—of having in the past consulted a psychiatrist, even for anxiety or some emotional problem—may be used to substantiate the standard diagnosis of schizophrenia under which such dissidents are committed. Indeed, says Mr Fainberg, the psychiatrist who is to report on a given dissident is sometimes selected on the basis of such background evidence. If a dissident happens to be the child of a broken marriage, for example, he will be sent to a psychiatrist who is particularly interested in that field.

Asked if any of the detainees referred for a psychiatric report are ever returned to the KGB certified sane, Mr Fainberg observed that in such cases "experienced psychiatrists know what is expected of them" and that if, on rare occasions, someone in the provinces does file a negative report, a second opinion will be sought. The only exceptions have been in response to world opinion, as in the Medvedev case or that of Vladimir Boukovskii. Bukovskii, a former biology student who was expelled from his institute before graduation, has had a long history of dissidence and had already spent some time in a penal psychiatric institution when, in 1971, he was arrested for publicising the cases of General Grigorenko and of Mr Fainberg himself. Bukovskii was sent by the Court to the Serbskii Forensic Psychiatric Institute in Moscow for obser-

vation but it so happened that, just at that time, the World Congress of Psychiatrists was taking place in Mexico. It is to Soviet fears of the possible reaction of the assembled psychiatrists that Mr Fainberg attributes the fact that Bukovskii was returned by the Serbskii psychiatrists to the court.

Once in the penal mental hospital, says Mr Fainberg, the dissidents are generally isolated from other patients. Conditions are harsh (one hour of exercise per day, permission to write to relatives only once a fortnight, and then under supervision). 'Treatment' consists largely of massive doses of aminazine (chlorpromazine) or haloperidol, far in excess of any legitimate therapeutic dose, given orally if possible or else by injection. Patients who refuse the tablets, he says, are beaten and kicked by the male nurses—injuries being officially attributed to the patient "falling and hurting himself". The only way to refuse such compulsory medication is by the threat of hunger strike, since this, in the case of the more notable dissidents, can attract undesirable publicity. Fainberg says that the remarks of certain of the staff indicated that they do not accept the official diagnosis, and that the dissidents themselves counter all attempts to convince them of their own insanity by speaking only to those members of the staff who do not consider them mad.

The pattern of treatment of dissident patients varies, it would seem, throughout the Soviet Union, being most severe in outlying areas. The Dnepropetrovsk institution, where the unfortunate Chinov was subjected to 30 insulin shocks and to electroconvulsive therapy (ECT) before his relatives managed to get him transferred to Leningrad, has a particularly bad reputation. In 1972, it was proposed to disperse all the dissidents from Moscow and Leningrad to institutions in remote provinces—a policy which led Viktor Fainberg to embark on a hunger strike (his fourth) and to smuggle out an appeal to Kurt Waldheim. This dispersal policy was then abandoned.

Soviet psychiatric theory, based as it is on Pavlovian behaviourist ideas, is peculiarly amenable to the concept that dissidents can be turned by psychiatric measures back onto the paths of correct Marxist-Leninist thought. But it would seem from Mr Fainberg that the Soviet use of psychiatry as a means of dealing with dissidents cannot be explained as a sincere attempt, however misguided, at the therapy of persons genuinely regarded as deviant from the social norm, but is rather a deliberate and cynical abuse of professional skill.

correspondence

Moon matters

SIR,—If your editorial "Bringing men to the Moon" (October 25) had been that of any journal other than *Nature*, we should have ignored it. In view of your prestige and of your great influence within the scientific community, we have decided to answer it.

You have sought to discredit this conference on a number of counts. You write, in reference to a previous conference, "while the scientists thought it silly, the philosophers took it all very seriously". In fact, at this meeting, only two of the 20 scholars were philosophers.

The purpose of the International Conference on the Unity of the Sciences is to further the field of communication between science and philosophy. The response of scientists to matters are naturally different from that of the philosophers. This indicates ignorance of modern philosophy.

Your sarcastic reference to the Second Conference in Tokyo is most surprising, since the conference itself was a success. Proceedings are available to those interested.

As to the 'messages of congratulation' by Nobel Laureates—messages of good-will, you must admit, can only be sent by the free will of those who send them. The manner of your reference to this indicates a prejudice. To write in this vein is nothing less than to despise a person, whoever he may be.

You attribute the most sinister possible motives to the founder of the organisation under whose auspices this conference is taking place. Surely Mr Moon, as any other philosopher of science living in the free world, is quite free to be devoted to any philosophy that he may choose for himself. Would it be unnatural to think that the initiator of the International Conference holds a specific point of view.

The aims, purposes and the nature of the Foundation, as admirable as they are, as set forth in the conference brochure, were clear and precise. Unfortunately, however, your statements about it seem to suggest that you had never read it.

You say that not all the advisers were very pleased to have their names used in this way. Basically, we have no intention of using the names of eminent scholars for purposes contrary to their own wishes. As you may know one person who agreed early on to

serve as an adviser subsequently chose to withdraw for his own reasons. As to our other advisers, both in the United Kingdom and internationally, they contributed greatly to the preparations of this significant event.

A feature of your editorial, unfortunately, is that general statements, even parts based on objective fact, demonstrate the prejudice and the apparent ill-intentions of the writer.

The list of participants you refer to was printed several months ago and included several names among which were firm acceptances as well as tentative ones. You have used this document to make allegations of improper conference management.

In reply to your enquiry: as founder of the Foundation under whose auspices this conference is taking place, Mr Moon has a right to be present and, contrary to your suggestion, he will, I understand, actually deliver the Founder's address.

It is suggested that in future you avail yourself of a more rigorous methodology for the purpose of discrediting those scientific enterprises of which you disapprove.

Morals are but an attempt to examine goals. And how can any behaviour be judged scientifically except in terms of its ability to achieve a goal? Any other criterion you must admit can only be purely subjective.

It may be true that there is, at present, no scientific methodology for the examination of basic goals, but the fault is surely with modern science for not having developed it. For until it is developed, scientists must accept their incapacity to examine the very assumptions on which their work is based. They must content themselves with doing things, without ever knowing why they should be done.

Contrary to what you intimate, we have no ideological bias. A number of scientists from the Communist countries were invited to this conference but, as it happened, could not attend.

It is suggested by way of conclusion that you ought to have taken the trouble to meet the person of central responsibility for the activity in question rather than questioning a subordinate employee whose knowledge may well be limited and even inaccurate.

In this case, I encourage you to accept the invitation extended to you in June this year, and to attend the Third International Conference on the

Unity of the Sciences, in order that you may evaluate its true value and import first hand, rather than relying on second-hand and perhaps unreliable sources.

In your allusion to 'their Boeing complex and their propensity to amateur philosophising', in reference to the eminent scientists, you have intentionally or unintentionally demonstrated your contempt and disrespect for those "deeply concerned scholars" who are willing to contribute to the urgent issues of the unity of science and the relation between science and morals.

P. BRIAN WIJERATNE

ICUS, London SW1, UK

The 'subordinate employee whose knowledge may well be limited and even inaccurate' was the 'Chief Public Relations Officer.'—ED.

Chemicals and cancer

SIR,—Your October 11 issue summarised some of the issues in the Aldrin and Dieldrin Suspension Hearing.

I was attributed—incorrectly—with five criteria which must be met before there is sufficient evidence of human carcinogenicity. These criteria appeared in the Administrator's Opinion of October 1, and are not quoted from any statement made by me. An EPA attorney and an EPA witness caricatured my opinions by suggesting that I would only recommend the removal of a product from the market after cases of human cancer had occurred. This is not—and never has been—my opinion. To avoid any misunderstanding I submitted an additional statement on this topic to the hearing record on September 11, in which I also indicated that their belief was apparently based on my answer to the question whether we would take dieldrin off the market if human cancer cases occurred.

My affirmative answer did not imply that I would not give advice to that effect at an earlier stage. It is not my position that we must wait for human cancer before removing a product from the market.

I was, therefore, surprised to see in the Administrator's Opinion that my "demands are practically impossible to meet".

D. E. STEVENSON

*Tunstall Laboratory,
Sittingbourne, Kent, UK*

news and views

Vaccination against malaria

ATTEMPTS to find a vaccine against malaria began in the 1930s but gave way to searches for new drugs during the war and anti-mosquito programmes after it. The combined use of anti-malaria drugs and insecticides has been one of the success stories of this century. The number of people infected with malaria has decreased considerably over the past decade in spite of the fact that, because of the increasing world population, more people than ever before now live in malarious areas. But resistance to drugs and insecticides, lack of money and political instability have reduced the future prospects of malaria eradication and thoughts have again turned to the possibility of developing a successful vaccine.

Three different sorts of vaccine are at present being investigated: irradiated sporozoites from the mosquito, extracts from schizonts (developing stages in the blood) and emulsified merozoites (the stages which pass between blood cells). After promising results using irradiated sporozoites in rodents, an experiment with *Plasmodium falciparum* in humans has resulted in one of three volunteers being protected against homologous (Clyde *et al.*, *Am. J. Med. Sci.*, **226**, 169; 1973) and heterologous strains of the same parasite (Clyde *et al.*, *Am. J. Med. Sci.*, **266**, 398; 1974). Earlier this year Simpson, Schenkel and Silverman (*Nature*, **247**, 304; 1974) succeeded in protecting rhesus monkeys against *Plasmodium knowlesi* using non-viable fractions extracted from disintegrated parasites. This and earlier studies, taken together, have shown that eleven out of seventeen vaccinated monkeys survived challenge whereas eight controls did not. Both these kinds of approach obviously have some potential promise but they still need a considerable amount of development.

The most promising vaccination results so far achieved with monkeys are reported in this issue of *Nature* by Mitchell, Butcher and Cohen from Guy's Hospital Medical School. They used *Plasmodium knowlesi* and their vaccine consisted of emulsified cultured merozoites. Six monkeys were immunised in various ways and challenged some time later with the homologous parasite or heterologous variants. Protection against the homologous parasite was absolute in two monkeys and strong in the third. There was also considerable protection against heterologous variants and the parasitaemias never rose above 1.5% whereas control animals died after about a week. Subsequent challenges with other variants resulted in equally encouraging results. The merozoite vaccine was found to be species specific and afforded only minimal protection against challenge with *Plasmodium cynomolgi bastianellii*.

These results show that the merozoite vaccine induces immunity better than that obtained after repeated challenges and cures. This contradicts the belief that vaccination against malaria cannot induce an immunity better than that which can be acquired naturally. The fact that the immunity induced transcends challenge with several variants suggests that the antigenic variation that occurs in malaria is not an insuperable barrier to vaccination, as has already been shown by Clyde and his co-workers. Of particular interest is the fact that the merozoite vaccine is simple to prepare and that 1 ml of parasitised blood cells provides twenty

immunising doses. These are early days yet and the next step will have to be the evaluation of the merozoite vaccine in owl monkeys, which are the only suitable primates that can be infected with human malaria. These monkeys are becoming increasingly difficult to obtain as thousands have been used for testing drugs. Very soon malariologists will have to draw up priorities for the use of owl monkeys and the whole drug/vaccine argument will have to be thrashed out afresh.

F. E. G. Cox

Messenger RNA doing without poly(A)

ONE of the tenets of cell biology has been that all messengers in the cytoplasm of eukaryotic cells (with the sole exception of the histone mRNAs) contain a length of poly(A), which fulfils some essential, although as yet unidentified function. When polysomal mRNA is examined for poly(A) content by reaction with poly(U)-sepharose or oligo(dT)-cellulose, most is bound; the fraction that is not retained has generally been thought to result from breakages in mRNA during isolation—since there is only one length of poly(A) in each mRNA, a single breakage would release a molecule altogether lacking poly(A). In two quite different systems, however, it now seems that an appreciable proportion of the cellular mRNA may lack poly(A). Working with HeLa cells, Milcarek, Price and Penman (*Cell*, **3**, 1–10; 1974) find that about 30% of the mRNA lacks poly(A); and using early sea urchin embryos of two species (*Strongylocentrotus purpuratus* and *Lytechinus pictus*), Nemer, Graham and Dubroff (*J. molec. Biol.*, **89**; 1974) report that about 40% of the messages may lack poly(A).

To examine mRNA of HeLa cells, it is necessary both to prevent labelling of ribosomal RNA and also to exclude contaminants derived from the non-messenger RNA population. The second can be achieved by releasing mRNA from polysomes with EDTA and in these experiments the first condition was met by using fluorouridine to suppress labelling of rRNA. The labelled mRNA fraction was then passed through a column of oligo(dT)-cellulose under conditions in which more than 96% of the poly(A)-containing mRNA is retained. This divides the mRNA into a major (70%) fraction containing poly(A) and a minor (30%) fraction apparently lacking it. Both poly(A)⁺ and poly(A)[−] mRNA have the same size distribution. That both classes of mRNA are in use in the HeLa cell as templates for protein synthesis is suggested by the use of puromycin, which releases both from the polysomes.

Only the demonstration that the nucleotide sequences of poly(A)⁺ and poly(A)[−] mRNA fractions are different can provide satisfactory evidence that they represent two genuine cellular species and that the poly(A)[−] is not in fact derived from the poly(A)⁺. The sequences of the poly(A)⁺ mRNA can be scrutinised by production of a complementary cDNA probe through the reverse transcription mediated by the enzyme of RNA tumour viruses; and in hybridisation

What controls rates of evolution?

EVER since the pioneer work of G. G. Simpson some three decades ago it has been recognised that one of the most important contributions that palaeontology can make to our knowledge and understanding of evolution is in determining rates of morphological change for different fossil groups over long periods of time. Simpson was the first to bring comparative quantitative data to bear on this subject, and convincingly demonstrated that, in terms of rates of origination and extinction, and of structural innovation, the mammals easily led the field. Van Valen has now endeavoured to show, in a letter published on page 298 of this issue, that whereas in general mammals have evolved at a faster rate than other organisms, there are at least two types of evolution in which the rate is effectively the same, namely the rate of amino acid substitution in proteins and change of size measured linearly. The first type of evolution is termed the epistandard, the other the standard mode.

Van Valen attempts a tentative explanation of the epistandard mode in terms of his Red Queen hypothesis, which is spelled out fully in an earlier paper (*Evolution Theory*, 1, 1; 1973). Readers of *Through the Looking Glass* will recall that the Red Queen explained to Alice that it took all the running one can do to stay in the same place. Van Valen put forward his hypothesis in an attempt to explain why the probability of extinction of fossil groups was more or less constant in time. It is based on the idea that all species within a given adaptive zone compete intensively. A successful adaptive response by one species is assumed to occur at the expense of other species, which must either adapt by themselves speciating or become extinct, as the 'quality' of their environment is reduced. This phenomenon leads to an endless chain of adaptive responses and in the long run

means that fitness and rate of extinction remain constant.

The high rate of diversification and evolutionary turnover in mammals is likely to be the result of a variety of factors, such as strong competitive interactions leading to specialisation in feeding methods, limitations on food supply, high mobility and energy use, interspecies aggression and territoriality. Such factors will conspire to lower the 'resource threshold' needed to prevent extinction, compared with other animals. Epistandard rates are required to make up the losses through extinction.

Though the Red Queen model might apply well to mammals, there are doubts about its more general validity. This is brought out well, for instance, in a thoughtful paper by Stanley (*Systematic Zool.*, 22, 486; 1973), who analyses the effects of competition on evolutionary rates. Attention is confined mostly to the mammals and the bivalve molluscs (that is, clams and oysters). In sharp contrast to mammals, the bivalves are nearly all sea-bed suspension feeders which mind their own business, characterised by weak interactions with other species, primitive inflexible behaviour, uncrowded, largely sedentary mode of life and generalised feeding habits.

This has as a direct consequence substantially lower rates of evolution than mammals, as Stanley amply documents. Limits on the bivalve populations are imposed more by predation and fluctuations in the physical environment than by food resources, and biological competition is minimal. As Stanley observes laconically, "Interspecific aggression is not characteristic of bivalve behaviour". What is true of bivalves is without much doubt true of the majority of benthonic invertebrates. *Through the Looking Glass* might be less appropriate as a textual source for the relevant evolutionary sermon than *The Walrus and the Carpenter*. A. HALLAM

experiments, the cDNA anneals back to its poly(A)⁺ mRNA template, but does not react with the poly(A)⁻ mRNA until much higher *R_{0t}* values are reached (and perhaps only then because of the presence of contaminating poly(A)⁺ mRNA in the poly(A)⁻ fraction). Because the cDNA represents only the 500 or so bases at the 3' terminal end of the messenger, immediately adjacent to the poly(A), the hybridisation analysis does not in itself exclude the possibility that the poly(A)⁻ mRNA represents 5' ends broken off poly(A)⁺ messengers; but this is rendered rather unlikely by the observation that poly(A)⁺ and poly(A)⁻ messengers are of the same size.

A similar situation is found in early sea urchin embryos, in which the sequences present in the poly(A)⁺ and poly(A)⁻ mRNA fractions were investigated by preparing a cDNA from the poly(A)⁺ mRNA by using the DNA polymerase I enzyme of *Escherichia coli*. At the maximum *R_{0t}* values reached, some 80% of this cDNA annealed with the poly(A)⁺ mRNA; the low level of reaction with the poly(A)⁻ mRNA shows that this fraction is almost entirely constituted of sequences different from those present in the poly(A)⁺ mRNA class. From the hybridisation reaction between the cDNA and poly(A)⁺ mRNA, it is possible to calculate that the complexity of the poly(A)⁺ mRNA corresponds to about 1,400 different species, each present on average 10,000 times in the embryo (which at this stage corresponds to about 400 cells). The complexity and abundance of the poly(A)⁻ class cannot be estimated since no probe for its sequences has been developed. Since the earlier work of Galau *et al.* (*Cell*, 2, 9-22; 1974) showed that the total polysomal mRNA falls into two abundance classes at the 600 cell stage, with less than 10% of the

mRNA representing 14,000 sequences present on average 340 times in the embryo, while most of the mRNA represents a much smaller number of sequences present in many copies each, it is of obvious importance to define the complexity of the poly(A)⁻ mRNA and to see how the total messenger population is divided into classes containing and lacking poly(A).

Only the histone messengers previously have been shown to lack poly(A), a feature which it was thought might be related to their synthesis during only part of the cell cycle; and also which it seemed possible might be responsible for their rapid appearance in the cytoplasm after addition of a radioactive label—the delay in appearance of a label in poly(A)⁺ mRNA might be due to the time involved in addition of poly(A). The kinetics of production and decay of the poly(A)⁺ and poly(A)⁻ mRNA classes in the HeLa cell, however, seem to be indistinguishable, so that whatever mechanism is used to produce the poly(A)⁻ class does not promote a more rapid cytoplasmic transport. Nothing is known yet about the mechanism of production of poly(A)⁻ messages, whether a large precursor is involved as seems to be the case with poly(A)⁺ mRNA or whether some different mechanism is implicated. The existence of poly(A)⁻ mRNA, however, appears to argue against models which suppose that the addition of poly(A) provides the only mechanism by which messengers are recognised for transport to the cytoplasm; and also raises the question of what function the poly(A) may play in the cytoplasmic activities of the messengers containing it. The purpose of poly(A) is therefore now less clear than ever.

BENJAMIN LEWIN

Alpha-particle transfer

from P. E. Hodgson

NUCLEAR reactions in which a nucleon or group of nucleons are transferred from the incident particle to the target nucleus are one of the most fruitful sources of information about nuclear structure. Single-nucleon transfer reactions have been extensively studied and have provided detailed information about the single-particle states of nuclei. The mathematical description of such processes is much more complicated when several particles are transferred, but already much work has been done on two-nucleon transfer reactions. Reactions in which an alpha particle is transferred are also being extensively investigated, and since the alpha particle is tightly bound it is sometimes a good approximation to treat it as a single particle and to use the formalism of single-particle transfer reactions.

Nuclear reactions initiated by heavy ions often show simple features that can be quite well understood by a semi-classical theory. Thus we can think of the incident particle following a definite orbit as it is scattered by the target nucleus, as a comet is deflected by the gravitational field of the Sun. If a transfer of nucleons takes place it naturally does so when the orbit of the incident particle is nearest the surface of the target nucleus (see Fig. 1).

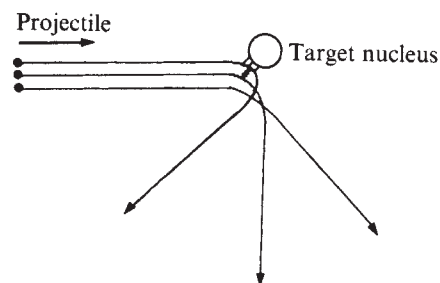


Fig. 1 Sketch showing the relations between the impact parameters, the scattering angles and the probability of a reaction taking place for several different classical orbits.

If now we consider such classical orbits of steadily decreasing impact parameter (the distance of the closest approach in the absence of a deflecting field) we can easily see that those with large impact parameters pass so far from the nucleus that a transfer reaction is very unlikely. Since these orbits are not strongly deflected, the cross section for particle transfer is small for small scattering angles. As the impact parameter is reduced the scattering angle increases and the orbit approaches nearer to the nuclear surface, increasing the likelihood of a transfer reaction. Thus the cross section of the transfer reaction initially

increases as the scattering angle increases. This increase continues until the orbit just touches the nuclear surface, and in this case the transfer is most likely. If the impact parameter is still further reduced the orbit cuts through the nucleus and the projectile interacts so strongly with the target nucleus that a very large number of reactions can take place and the probability of the simple transfer reaction falls. Thus on the simple classical picture we expect the transfer reaction to have a cross section that first rises to a maximum and then steadily falls as the scattering angle is increased.

Such bell-shaped cross sections have frequently been found in studies of heavy ion transfer reactions, and semi-classical calculations give good agreement with the experimental data. These calculations are called semi-classical because the projectile is assumed to move along a classical orbit in the field of the target nucleus, but the actual transfer process is treated quantum-mechanically.

A fully quantum-mechanical theory of these reactions has also been developed, and it is known as the distorted wave theory. The interacting particles are represented by wave functions, and the way they are distorted by the field of the target nucleus is found by solving the appropriate Schrodinger wave equation. Such calculations also give the bell-shaped curve, and also some of the details of the cross sections that are not given by the classical theory (see *Nature*, **246**, 334; 1973).

Last year the differential cross section of the alpha-particle transfer reaction $^{32}\text{S}(^{16}\text{O}, ^{12}\text{C})^{36}\text{Ar}$ was measured by Braun-Munzinger and colleagues in Heidelberg (*Phys. Rev. Lett.*, **31**, 1423; 1973) and they found that contrary to expectation it has a strongly oscillatory structure, as shown in Fig. 2. Calculations of the cross section of this reaction gave the usual bell-shaped curve, so it was an important challenge to try to understand the origin of the pronounced oscillations that were observed experimentally.

Charlton and Robson (*Phys. Rev. Lett.*, **32**, 946; 1974) tackled this problem and found that if it was assumed that the transferred alpha particle was in its 2^+ state at 28.5 MeV an oscillatory angular distribution is obtained, in good overall agreement with the experimental data. Although this process may seem somewhat unphysical due to the high excitation energy required, detailed nuclear structure calculations showed that the probability of the dissociation $^{36}\text{Ar} \rightarrow ^{32}\text{S} + \alpha^*$, where α^* stands for the excited alpha particle, is such that the transfer for the excited alpha particle

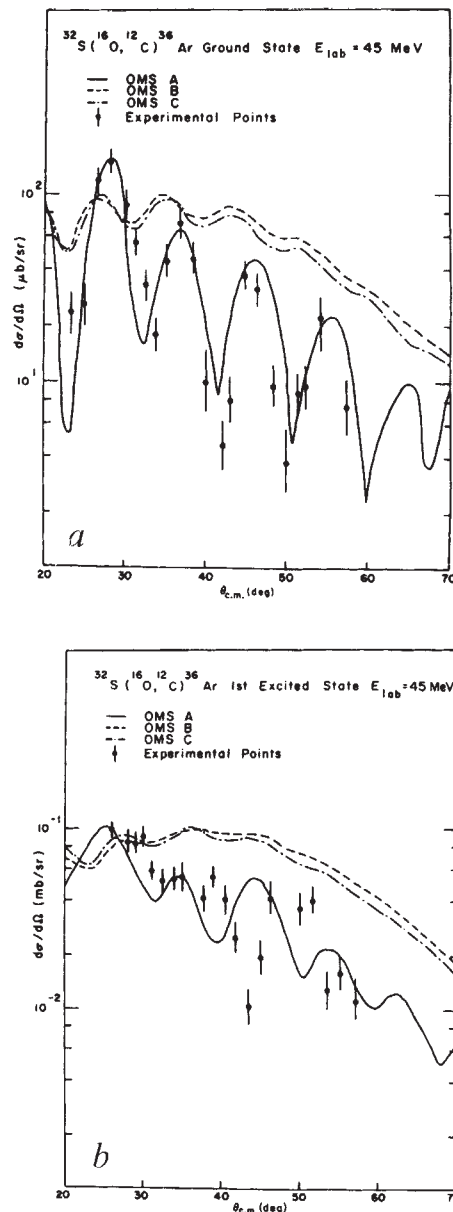


Fig. 2 Differential cross sections for the alpha-particle transfer reaction $^{32}\text{S}(^{16}\text{O}, ^{12}\text{C})^{36}\text{Ar}$: a, ground state; b, first excited state, at 45 MeV compared with distorted wave calculations with the three sets of potentials in the table.

is about five times more likely than transfer in its ground state.

Additional studies of this reaction have recently been made by Werby and Tobocman (*Phys. Rev.*, **C10**, 1022; 1974) and they investigated in detail the effect on the cross section of varying the potential that represents the interaction between the incident particle and the target nucleus. This is in fact not known very well, and what is usually done is to assume that it has the form of an optical model potential and to adjust its parameters to give a good fit to the differential cross section for the elastic scattering interaction, when the incident particle is simply deflected without losing energy or nucleons. Unfortunately the potentials found in this way are not

unique, and it is possible to find several sets of parameters that give equally good fits to the elastic scattering data. This is particularly so at low energies where the elastic scattering cross section is rather featureless, as is the case for the reaction of interest here.

Werby and Tobocman found several quite different potentials that all give acceptable fits to the elastic scattering, as shown in Fig. 3. The first of these labelled A has an absorbing potential of only 6 MeV, much less than the 30 MeV used by Charlton and Robson; all the parameters of these potentials are given in the table.

The cross sections for the alpha-transfer reaction calculated with these potentials using the distorted wave theory are shown in Figure 2a, and it is immediately evident that the strongly absorbing potentials give curves with weak oscillations that have the overall bell shape characteristic of the classical case, while the weakly absorbing potential A gives a strongly oscillating curve that agrees very well with the experimental data. Since these calculations assumed that the transferred alpha particle is in its ground state, it is not necessary to postulate the transfer of excited alpha particles in order to obtain a good fit to the data.

These results all refer to the reaction that leaves the residual nucleus ^{36}Ar in its ground state. Experimental data are also available for the same reaction to the first excited state, and the corresponding measured and calculated cross sections are shown in Figure 2b. The experimental cross sections again show an oscillatory structure, though it is not so marked as for the reactions to the ground state, and once again the strongly

TABLE Optical model parameter sets. $V = (V_0 + iW)\{1 + \exp[(r - R)/a]\}^{-1}$, $R = r_0(A_1^{1/3} + A_2^{1/3})$.

Channel	Set	V_0 (MeV)	W (MeV)	r_0 (fm)	A (fm)
$^{16}\text{O} + ^{32}\text{S}$	A	40	6	1.3	0.5
$^{12}\text{C} + ^{36}\text{Ar}$		40	4	1.3	0.5
$^{16}\text{O} + ^{32}\text{S}$	B	40	24	1.3	0.5
$^{12}\text{C} + ^{36}\text{Ar}$		40	16	1.3	0.5
$^{16}\text{O} + ^{32}\text{S}$	C	100	30	1.22	0.5
$^{12}\text{C} + ^{36}\text{Ar}$		100	30	1.22	0.5

absorbing potentials give a featureless bell-shaped curve while the weakly absorbing potential gives an oscillatory cross section in qualitative accord with the data.

It is interesting to try to understand just why the weakly absorbing potential gives an oscillatory cross section while the strongly absorbing potentials do not. This can be done by examining the details of the formalism of the distorted wave theory, in particular the contributions of the different partial waves to the reaction amplitude. It is found that relatively few partial waves contribute, especially when there is no angular momentum transferred in the reaction, as is the case for the present reaction to the ground state of ^{36}Ar . Now the contribution of a particular partial wave depends on an integral of the products of the radial parts of the distorted waves of the incident and outgoing particles and a form factor depending on the positions in space of the colliding particles. The value of this integral depends on the potentials chosen, and then what determines the overall features of the cross section is the way the phase of the integral varies from one partial wave to the next for the important partial waves. It is found that for the weakly absorbing potential A the phase remains fairly constant in this region, giving constructive interference between the integrals for the different partial waves. For the strongly absorbing potentials B and C, however, the phase of the integral changes rapidly and produces destructive interference in the critical region. This difference in the behaviour of the integrals is responsible for the difference in the cross sections corresponding to the two types of potentials.

In the case of the reaction to the first excited state of ^{36}Ar , two units of angular momentum are transferred and this increases the number of partial waves that contribute significantly to the interaction. This has the effect of reducing the amplitude of the oscillations, but the qualitative explanation of the difference between the cross sections calculated with the two types of potentials is still valid.

It should be noted that the calculated cross sections in Fig. 2 have been normalised to the experimental data. The value of the normalisation factor depends on the product of the spectroscopic amplitudes for the dissociation

of the incident ^{16}O into $^{12}\text{C} + \alpha$ and the combination of α and ^{32}S to give ^{36}Ar . If excited alpha particles are also transferred the amplitudes for the corresponding dissociation and combination have also to be included in the calculation. At present the nuclear structure part of the process is not understood sufficiently well for reliable quantitative calculations to be made, and it will be a critical test of such calculations to see if they give the measured values of the cross sections.

It is clear that in spite of considerable progress there is still much to be learnt about the mechanism of these alpha-particle transfer reactions. We do not know what potential to use for the interaction between the incident particle and the target nucleus, and it is likely that further progress will come from the many attempts that are now being made to calculate the potential from a detailed theoretical model of the interaction, instead of the phenomenological approach used in the present calculations. It is clear that the elastic scattering alone does not determine the potential accurately enough, but studies of reactions such as those described here enable it to be more precisely defined. Thus it may be that studies of a wide range of reactions will also give more reliable potentials. On the nuclear structure side there is also much work to be done, and this should enable the relative contributions of unexcited and excited alpha-particle transfers to be evaluated.

The present situation for these reactions frequently occurs in nuclear reaction calculations. At first there is a strange cross-section that disagrees with existing calculations. Then a new model is developed that seems to explain the results, until it is found that several different models all do equally well. At this stage it is necessary to probe deeper and try to understand what is going on in a very detailed way, so as to distinguish between the merits of rival models.

Movement of auxin across plant cell membranes

from our Plant Cell Physiology Correspondent

AUXINS stimulate the extension growth of plant cells. They are synthesized in the shoot apex and regulate the expansion of cells below the tip. Studies with the naturally occurring auxin indole acetic acid (IAA) and the synthetic analogue 2,4-dichlorophenoxy-acetic acid (2,4-D) have shown that these compounds move preferentially from the apex towards the base of plant stems. This process is often referred to as

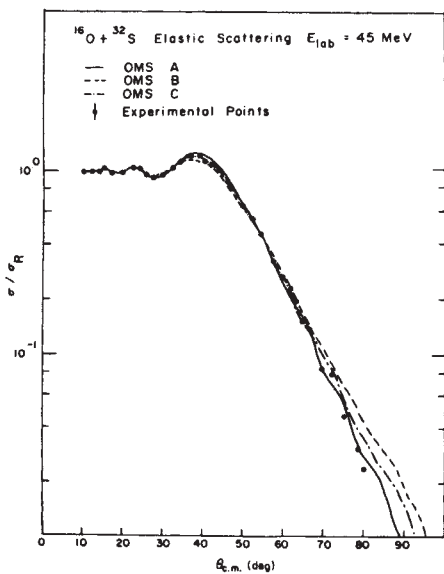


Fig. 3 Differential cross section for the elastic scattering of 45 MeV ^{16}O by ^{32}S compared with optical model calculations with the three potentials given in the table.

'active transport' because it requires energy, and the movement of applied auxin from base to apex of stem segments is extremely low by comparison. The continued production and export of auxin from the shoot tip is thought to be responsible for such phenomena as the inhibition of outgrowth of lateral buds by the apex and the control of adventitious root production at the base of cut stems. For these reasons studies on the polar transport of auxin and the mechanism of auxin movement across the plasmalemma are of considerable interest.

Recent experiments by Rubery and Sheldrake carried out in the University of Cambridge have shown that there

are at least two different mechanisms for the passage of auxin across membranes and that 'secretion' of auxin from cells may not be driven directly by the hydrolysis of ATP but rather may depend upon the pH gradient across the membrane (*Planta*, **118**, 101; 1974).

Rubery and Sheldrake used crown gall cells of Virginia creeper grown in suspension culture as a model system to study auxin influx and efflux under a variety of experimental conditions. (Unlike most cultured plant cells crown gall cells do not require an exogenous supply of auxin in order to grow.) In a previous paper (Rubery and Sheldrake, *Nature new Biol.*, **244**,

288; 1973) it was shown that with the external medium at pH 4.3, when IAA is only slightly ionised ($pK=4.7$) there is a linear relationship between IAA uptake and the external concentration up to 500 μM IAA. Under these conditions it is assumed that the undissociated IAA, which is lipid soluble, enters the cells by diffusion. Once inside the cells where the pH of the cytoplasm is above pH 7, IAA would be expected to ionise, thus maintaining a concentration gradient for the passage into cells of undissociated IAA by diffusion. The authors now suggest that a passive anion carrier also operates between pH 5 and pH 6.7. The optimum for the anion carrier is about pH 6 and the carrier is apparently half saturated at quite low IAA concentrations within the range 1–5 μM . The carrier is capable of bringing about either the influx or efflux of IAA anions depending upon the direction of the concentration gradient. Studies on the operation of the carrier system were greatly aided by the observation that 2,4-D inhibits the carrier-mediated IAA anion influx and that 2,3,5-triodobenzoic acid (TIBA), a known inhibitor of polar auxin transport, prevents the efflux of anions but has no effect upon the entry of IAA anions into cells. These results suggest that the properties of the carrier are different on the inside and outside of the plasmalemma and that 2,4-D binds to the carrier at the external face and TIBA binds at the internal face of the membrane. The apparent specificity of TIBA in inhibiting anion efflux is very interesting because it has been known for some time that in shoot segments TIBA inhibits polar auxin transport by interfering specifically with the exit of IAA from the cells.

Rubery and Sheldrake point out that if an auxin carrier similar to the one that operates in crown gall cells is localised in the membrane at the basal end of the cells in plant shoots, this would provide a mechanism for the observed polar transport of IAA. When the pH within the cell walls is acidic, auxin will enter the cells by diffusion and accumulate there. Eventually the internal anion concentration will rise above that outside the cell and the carrier will mediate the efflux of IAA anions across the membrane at the base of the cell into the cell wall. Providing the pH within the wall is lower than that of the plant cytoplasm some of the auxin will then enter the adjacent cell by diffusion where it will again be carried across the basal membrane as IAA anions. The net result of this process is polar auxin transport. Although ATP is not required to drive the secretion of IAA across the plasmalemma there is an indirect requirement for energy in order to maintain

Density of galactic space

from David W. Hughes

THE mean density of space in the solar region of the Milky Way galactic plane is an important astrophysical quantity. First it is a fundamental parameter in the study of galactic dynamics and second it gives a lead to the way in which the galactic material is proportioned out between stars, gas and dust.

By studying the variation of galactic rotation as a function of distance from the nucleus Schmidt (*Galactic Structure*, University of Chicago Press, 1965) calculated that the total local mass density near the Sun should be $0.14 M_{\odot} \text{ pc}^{-3}$, where $M_{\odot}$ is the solar mass ($2 \times 10^{33} \text{ g}$) and a parsec is $3.08 \times 10^{18} \text{ cm}$. This is equivalent to $1 \times 10^{-23} \text{ cm}^{-3}$. A similar value ($\sim 0.20 M_{\odot} \text{ pc}^{-3}$) was required by Oort (*ibid.*) to account for the observed stellar velocities perpendicular to the galactic plane. A problem immediately arose because the densities of stars, dust and gas in the vicinity of the Sun only added up to $0.084 M_{\odot} \text{ pc}^{-3}$ indicating either that the estimates of the individual densities were wrong or that the Galaxy contains some mysterious mass in the form of unobserved objects.

Luyton (*Mon. Not. R. astr. Soc.*, **139**, 221; 1968) calculated how the number of stars in regions of the Milky Way vary as a function of their luminosity, and using the mass-luminosity relationship found the stellar mass density to be $0.064 M_{\odot} \text{ pc}^{-3}$. The interstellar dust in the Galaxy can be detected by looking at the extinction of stars as a function of distance, the reddening of starlight that passes through dusty regions, the polarisation of starlight by intervening dust and finally by observing the diffuse galactic light. The mean density of

this dust is found to be $0.0015 M_{\odot} \text{ pc}^{-3}$. Interstellar gas produces atomic and molecular absorption lines in stellar spectra and also radiates emission lines in the microwave radio region of the electromagnetic spectrum. Measurements of these lead to a mean gas density of $0.018 M_{\odot} \text{ pc}^{-3}$.

The missing mass problem was partially solved in 1972 by Weistrop (*Astronomical Journal*, **77**, 849; 1972) who found that a substantial number of red dwarf stars had been overlooked previously and also that these stars are strongly concentrated towards the galactic plane. Weistrop's observations led to a 100% increase in the stellar mass density which then became $0.13 M_{\odot} \text{ pc}^{-3}$.

Glenn Veeder (Hale Observatories, California Institute of Technology) in a recent letter to the *Astrophysical Journal* (**191**, L57; 1974) has calculated a revised empirical relationship between the absolute visual magnitude of a star and its mass, this coming from observations of low-mass faint multiple stellar systems. Veeder finds that the main-sequence stellar mass density in the solar neighbourhood is now $0.16 M_{\odot} \text{ pc}^{-3}$. Add to this the mass contribution due to dust and gas and the density becomes $0.18 M_{\odot} \text{ pc}^{-3}$ with the material divided between stars, gas and dust in the percentages 89, 10, 1.

So the missing mass of 1965 has now been found and the results of the galactic dynamicists and the stellar astronomers agree within the expected margin of error. This also means that unobserved objects (black holes, dead white dwarfs and so on) must only make up a non-significant percentage of the galactic mass.

the pH of the cytoplasm above that in the walls. Providing that this requirement is met, the specificity of the transport system would reside in the preferential distribution of IAA anion carriers in the basal membranes of the cells. It is possible that an alteration in the distribution of the carrier might also explain the lateral movement of auxin observed during tropic responses.

Indicators of palaeoseismicity

from Peter J. Smith

DETERMINATION of the ancient geomagnetic field may be fraught with practical difficulties, but palaeomagnetists do at least have access to abundant material in which the field is recorded. Seismologists, by contrast, are less fortunate. Insofar as they are concerned with using seismic waves to investigate the Earth's internal structure and properties, they must be content with the Earth as it is today; in this sense there is no such thing as palaeoseismology. But it is possible to envisage the study of palaeoseismicity; and this branch of seismology already exists in a limited way. It is clear from plate tectonics, for example, that the bulk of the world's current seismicity coincides with the three types of plate boundary. And as the reasons for this correlation are broadly understood, it may reasonably be inferred that the association has always obtained. In principle, therefore, it should be possible to define the location and timing of palaeoseismicity by finding and dating ancient plate boundaries.

But this is a very general approach, equivalent in palaeomagnetism to defining when and where the Earth had a magnetic field. Is it possible to be more specific? Is there some phenomenon which could be used to determine the details of ancient seismicity or even the characteristics of individual seismic events, rather as rock magnetism may be used to determine the details of the ancient magnetic field? So far there is no known phenomenon which may be used to investigate palaeoseismicity in a routine way; but a hint that such investigations may ultimately be possible in certain situations has now come from Engelder (*J. geophys. Res.*, **79**, 4387; 1974) who has been studying the microscopic effects on surfaces which have slid against each other.

Engelder's suggestion is based on both laboratory and field studies. In the laboratory experiments, polished samples of Westerly granite were slid against each other for distances up to 3 mm and under confining pressures up to 2.9 kbar. At pressures below 0.3

kbar, stable sliding occurred with no damage to the polished surfaces. But at higher pressures stick-slip took place, producing wear grooves, scratches and small clumps of gouge on the surfaces. Of particular interest are the wear grooves which are generally carrot-shaped with the tip pointing in the direction of motion of the surface containing the grooves. The amount of slip can be calculated from the lengths of the grooves, for each event produces grooves with various lengths up to the distance of the slip (that is, groove lengths are not related to the total amount of slip where a series of individual slips is concerned but only to the distances of the individual slips themselves).

In the field, Engelder has found two natural counterparts to these experimental systems. The first is a slickenside (polished surface resulting from friction along a fault plane) in the Mesa Rica sandstone in the Bonita normal fault zone of New Mexico. In this case the longest of the carrot-shaped grooves are 5 mm long. Unfortunately, the total slippage during the formation of this slickenside is not known, so it is impossible to say whether the grooves were produced during a single slip event or during a series of events, although the grooves do point in the known direction of motion. In the second example, a slickenside in Tintic quartzite in the Eureka fault zone of Utah, neither the sense nor the total amount of slip is known. But if Engelder is right, the sense of slip may now be deduced from the grooves. Moreover, as the longest grooves here are 0.5 mm and as 0.5 mm of slip is insufficient to generate highly polished slickensides in quartzose rock, it may be concluded that more than one slip event, and probably such events, took place.

From the morphology and lengths of those natural and experimental grooves it is possible to deduce a mechanism for their formation. In simple terms, what apparently happens is that, at the initiation of a slip, interlocking asperities (which previously prevented slip) shear. As the slip progresses, other asperities (and gouge particles) gradually plough deeper into the opposite surface, forming grooves which gradually widen (as viewed in section) and slowing down the slip until it finally stops. The cycle is then repeated, beginning again with the shearing of asperities. If the asperities did not shear at the start of each slip but instead jumped out of their old grooves and ploughed new ones, a series of inline grooves would be formed. These are seldom observed. If, on the other hand, asperities failed to shear but instead continued to plough the old

grooves, grooves would generally be longer than one slip length. These are seldom observed either. What are observed, however, are some grooves which are shorter than one slip length, presumably because not all asperities begin to plough at the start of a slip.

Because Engelder is here dealing with microscopic features, the connection with palaeoseismicity is not direct. The point is, however, that some seismicity is clearly related to stick-slip events. Moreover, the mechanism summarised above closely resembles one previously put forward to explain macroscopic stick-slip behaviour. The question that Engelder poses is thus: is there a type of macroscopic groove on large fault planes that may be used for distinguishing seismic from aseismic faults, and for estimating the amount of slip during seismic events?

For the time being, this question cannot be answered. But if such grooves are found, Engelder's work, albeit on a microscopic scale, suggests that they could give a significant boost to the study of palaeoseismicity. Aki (*Bull. Earthquake Res. Inst.*, **44**, 73; 1966), for example, has proposed a formula for calculating an earthquake's seismic moment from the average displacement of one slip on the fault, a formula which Engelder uses to determine the moment of an event along the Bonita fault zone. A similar calculation could be carried out for macroscopic events along large faults, although the validity of such a scaling-up would first have to be tested rigorously.

Diseases caused by light

from a Correspondent

THERE have been advances in the past few years in knowledge of the photosensitive disease Xeroderma Pigmentosum. It was inevitable therefore that the British Photobiology Society meeting held on October 23 entitled "Recent Advances in Ultraviolet and Visible Radiation Effects on the Skin" should contain several papers dealing with this disease.

The opening paper, however, by Dr R. J. Murgatroyd (Meteorological Office, Bracknell) was of a much broader nature. He discussed the variations in stratospheric ozone and the changes in penetration of solar ultraviolet radiation which result from these variations. He stressed the large natural variation in stratospheric ozone and the lack of any consistent change with nuclear tests. Fears have recently been expressed that a reduction in ozone and a consequent increase in penetration of solar ultraviolet radiation would occur with supersonic aircraft flights. Dr Murgatroyd expressed the view in

Cattle help in conservation

from Peter D. Moore

ALTHOUGH conservation has come to mean much more than the simple preservation of rare species, the prevention of extinction of threatened plants and animals is still an important concern of the conservationist. Apart from the possibility of aesthetic loss (consider how great a contribution the giant panda has made to the fantasy worlds of our children), a depletion of the planet's genetic resources accompanies each extinction which may ultimately be regretted even by those who have lost their aesthetic awareness. A good example of a lowly plant made rare by the destruction of suitable habitats and which has received such attention from conservationists is *Dianthus armeria*, the Deptford pink, which is restricted to a few meadows in southern Britain (see Walls, *Proc. Bot. Soc. Br. Isl.*, 6, 337; 1967).

One of the sites at which this species occurs is Woodwalton Fen, a National Nature Reserve in the fenland area of East Anglia. Most of this nature reserve has been managed by man in the past, either for reed cutting, peat extraction or as grazing land; the area with *Dianthus armeria* was once a farmstead over which cattle were grazed. This practice was not continued in the early days of the reserve's history, but locally the pressure of rabbit grazing was sufficient to maintain the short-grass community in which *Dianthus* survived. Following the myxomatosis epidemic of 1954, however, extensive changes occurred in the vegetation of the area; coarse grasses, particularly *Calamagrostis epigejos* and *Agropyron repens*, spread at the expense of shorter-growing species, including *Dianthus*. By 1964 only five plants remained and at this stage a new management regime involving grazing by Friesian heifers was introduced. Subsequently the popula-

tion of *Dianthus* expanded gradually to 900 plants by 1967.

Williams, Wells and Wells (*J. appl. Ecol.*, 11, 499; 1974) have now attempted to analyse the respective roles of cattle and rabbit grazing in this particular recovery. They undertook a quantitative analysis of the faeces of these animals by concentrating the surviving plant epidermal fragments in a faecal sample and spreading them on a microscope slide. The species composition of these samples was subsequently estimated by means of randomly located point observations. This type of analysis was repeated periodically through the course of a year in order to determine the changing food preferences of the animals during that time. As Stewart (*J. appl. Ecol.*, 4, 83; 1967) has pointed out, there are many problems associated with this technique, particularly the relative digestibility of different species. But the preponderance of coarse grasses in the cattle faeces and the virtual absence of dicotyledonous species gives a reasonable representation of this animal's diet. The rabbits also seem to have neglected dicots, but show a stronger preference for the less coarse grasses, such as *Festuca*.

The results of these studies suggest that the beneficial effect of cattle upon the *Dianthus armeria* population results from their selective grazing upon the more aggressive species of grass. The techniques employed however, do not permit conclusions regarding the influence of trampling upon *Dianthus* by increasing microtopographical diversity. When scarce, the plant became restricted to ant hills, which suggests that soil disturbance and microtopographical factors may be important in controlling its distribution. Examination of the germination and establishment requirements of *Dianthus* under laboratory conditions might assist in the separation of these two distinct influences of cattle grazing. Meanwhile it cannot be denied that this is an efficient management practice for the conservation of a diminishing species.

The past few years have seen the emergence of Xeroderma Pigmentosum variants, patients with clinical features of the disease but with normal ability to carry out excision repair of DNA. Dr A. Lehmann (University of Sussex) has recently confirmed that in these patients all of the steps in excision repair are normal, but that there is a defect in post-replication repair. Post-replication repair in Xeroderma Pigmentosum variants is inhibited by caffeine, an effect which is not present in normal cells. Cells from patients with Xeroderma Pigmentosum who are defective in excision repair show changes in post-replication repair which are intermediate between normal and Xeroderma Pigmentosum variants.

Another aspect of this disease was discussed by Dr C. A. Ramsay (Institute of Dermatology, London). He demonstrated the abnormal reactions of the skin of these patients when they are irradiated with monochromatic ultraviolet light in the 290–320 nm region. The delay in the onset of erythema after irradiation may enable the diagnosis of Xeroderma Pigmentosum to be suspected early in life. In addition, Dr Ramsey reported the first successful case of the prenatal diagnosis of Xeroderma Pigmentosum by the detection of the deficiency of excision repair in cultured amniotic cells. Xeroderma Pigmentosum is the only condition to date in which excision repair of DNA has been found to be abnormal. Actinic keratoses occur on normal skin as a result of chronic exposure to solar ultraviolet irradiation and Dr J. Pitts (University of Glasgow) has found that in cells of such keratoses excision repair is not impaired.

Several other aspects of photosensitive skin disease were discussed. A review of the value of action spectroscopy in dermatology was given by Dr Frain-Bell (University of Dundee). The photodermatoses present many unsolved problems not least of which is that of treatment. Dr J. C. van der Leun (State University of Utrecht, Netherlands) has used the technique of repeated irradiation of the skin as a method of treatment and has found it of benefit in solar urticaria and polymorphic light eruption. These findings are of interest because the two diseases represent quite different reactions to sunlight; solar urticaria occurs very soon after irradiation whereas in polymorphic light eruption there is a delay of several hours before the skin changes occur.

Finally, a detailed study of the photochemical basis of a dye-induced photodermatitis was reported by Dr K. Davies (University of Salford). He showed that in this reaction with an anthraquinone dye, singlet oxygen is an important intermediate.

discussion that these fears have been exaggerated.

Recent work on the genetic basis of Xeroderma Pigmentosum have shown the value of studies of heterokaryons formed by fusing fibroblasts from patients with the disease and from normal subjects. Dr P. Lohman (Netherlands Organisation for Applied Scientific Research, Rijswijk) reported that there are now five complementation groups in Xeroderma Pigmentosum. Dr Lohman also discussed experi-

ments which showed that ultraviolet-irradiated chicken fibroblasts can repair DNA damage other than that involving pyrimidine dimers. Investigations by Dr F. Giannelli (Guy's Hospital Medical School, London) indicate that the enzyme which is defective in most patients with Xeroderma Pigmentosum is probably not a monomer. In addition, it is possible to detect heterozygotes for Xeroderma Pigmentosum mutations by the assessment of DNA repair in heterokaryons.

review articles

Do machines understand more than they did?

Yorick Wilks*

To be able to communicate in ordinary English, a machine must have access to common sense information about the real world. The first computer systems to attempt such communication lived in very limited worlds. Now, with a "second generation" of programs, those worlds are widening, although the exact significance of the recent advances is still in dispute.

IN his report to the Science Research Council on the state of artificial intelligence (AI), Sir James Lighthill¹ gave most of the field a rather bad prognosis. One of the few hopeful signs he saw was Winograd's computer system for natural language understanding². Yet now, only a year later, Winograd has stopped work on the system he constructed, and has begun a new one on entirely different principles. He went so far, in a survey lecture (Third International Joint Conference on Artificial Intelligence, Stanford, California, 1973) of extraordinary modesty in a field not known for its small claims, to place his celebrated early work in only the "first generation" of computer systems designed to understand natural language, before going on to describe others' "second generation" systems.

I shall return later to this metaphor of generations, but what is one to say in general terms of a field where yesterday's brightest spots are today's first generation systems, even though they have not been criticised in print, nor shown in any generally acceptable way to be fundamentally wrong? Part of the answer lies in the profound role of fashion in artificial intelligence in its present prescientific phase. A cynical American professor remarked recently that AI had an affair with someone's work every year or two, and that, just as there were no reasons for the falling in love, so, later, there were no reasons for falling out again. In the case of Winograd's work it is important now to resist this fashion, and re-emphasise what a good piece of research it was, as I shall show in a moment.

Another part of the answer lies in the still fundamental role of metaphysical criticism in AI. In the field of computer vision things are bad enough, in that anybody who can see feels entitled to criticise a system, on the ground that he is sure that his own vision is not based on the same principles. In the field of natural language understanding things are worse: not only does anyone who can speak and write feel free to criticise on the corresponding grounds, but those trained in disciplines parasitic upon natural language, linguists and logicians, often have strong views on *a priori* grounds on how things must be done. It is this metaphysical aspect of the subject that gives its disputes their characteristically acrimonious flavour.

In this article I want to sort out a little what is agreed and what is not; what are the outstanding disputes and what are the systems making the most testable claims. If what follows seems unduly philosophical, it should be remembered that little is agreed, and almost no achievements are beyond question. To pretend otherwise, by concentrating only on the details of established programs, would be meretricious and misleading.

Winograd's understanding system

Winograd's system runs as a dialogue, in real time, between a human operator who types in messages, and the system proper,

called SHRDLU, which displays on a screen pictures of a closed world of coloured blocks and pyramids, a box into which they can be put, and an arm or crane that can move the objects about. SHRDLU displays its replies to the operator by writing on the same screen. The following is a discontinuous sample of the dialogue Winograd gives in his book. SHRDLU's replies are in upper case:

pick up the big red block

OK

find a block which is taller than the one you are holding and put it into the box.

BY 'IT', I ASSUME YOU MEAN THE BLOCK WHICH IS TALLER THAN THE ONE I AM HOLDING

OK

what does the box contain

THE BLUE PYRAMID AND THE BLUE BLOCK

had you touched any pyramid before you put the green one on the little cube

YES, THE GREEN ONE

when did you pick it up

WHILE I WAS STACKING UP THE RED CUBE, A LARGE RED BLOCK AND A LARGE GREEN CUBE

why

TO GET RID OF IT.

This ingenious program was also able to accept new definitions of words, such as "steeple"; to make use of what it knew about the properties of the objects described, such as that a block could have its top cleared off, but a pyramid could not; and also to remember what it had done before, as in the sample above.

The program was written in the language PLANNER³, which is the concrete expression of the slogan "meanings are procedures", a sentiment into whose own meaning it is probably best not to enquire too closely, but which has undoubtedly led to a new style of programming. PLANNER is a theorem-proving language: it tries to establish the truth of assertions, not in the normal uniform, proof-theoretic, manner, but by accepting a range of "programmed hints" about how best to proceed at any point. In a language understanding program like Winograd's this means replacing familiar grammar rules such as:

S → NP + VP

(a sentence consists of a noun phrase followed by a verb phrase) by procedures, in this case:

((PDEFINE SENTENCE ((PARSE NP) NIL FAIL)((PARSE VP) FAIL FAIL RETURN)))

The details of the notation need not detain us; what is important is that Winograd's grammar is not the conventional list of rules, but small sub-programs like the line above, that actually represent procedures for imposing the desired grammatical structure. The definitions of more complex words are also in this form: here, for example, is the "theorem" defining the meaning of "pickup".

((DEF THEOREM TC-PICKUP (THCONSE (X (WHY (EV))EV)

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```
(#PICKUP $?X)(MEMORY)(THGOAL(#GRASP $?X)
(THUSE TC-GRASP))
(THGOAL (#RAISEHAND)(THNODB)(THUSE TC-
RAISEHAND))
(MEMOREND (#PICKUP $?EV $?X)))
```

Once again detailed explanation of the notation is not needed to make it clear that the word is being defined in terms of a number of more primitive sub-actions, such as RAISEHAND, each of which must be carried out in order that something may indeed be picked up. The linguistic content is a "systemic grammar"⁴; plus a simple system of semantic "features" marking words and arranged hierarchically, such as PHYSOB (for physical object words) and ANIMATE (for animate words like "robot"); together with some factual knowledge about the block world. Both types of knowledge, linguistic and factual, are represented in PLANNER and the program has access to whichever sort is required at any given moment, rather than proceeding in the conventional manner by first parsing to get a syntactic structure and then manipulating the features to get a semantic structure.

Significance and shortcomings of SHRDLU

One reason for the enormous impact of this work was that, before its appearance, AI work was linguistically trivial, while the systems of the linguists had no place for the use of inference and real world knowledge. Thus a very limited union between the two techniques was able to breed considerable results. Before Winograd there were few programs in AI that could take a reasonably complex English sentence and ascribe any structure whatever to it. In early classics of "natural language understanding" in AI, such as Bobrow's STUDENT⁵ problem solver for simple algebra, input sentences had to be short and of stereotyped form, such as "what is the sum of . . . ?" Conversely, in linguistics, there was, until very recently, little speculation on how we understand the reference of pronouns in such elementary sentences as "The soldiers fired at the women and I saw *several* fall," where it is clear both that the answer is definite, and that finding it requires some inferential manipulation of generalisations about the world. The reader should ask himself at this point how he knows the referent of the pronoun in that sentence.

So far, the reaction to Winograd's work has been wholly uncritical. What would critics find to attack if they were so minded? First, that his linguistic system is highly conservative, and that the distinction between "syntax" and "semantics" may not be necessary at all. Second, that his semantics is tied to the simple referential world of the blocks in a way that would make it inextensible to any general, real world, situation in which, for example, "block" might mean "an obstruction" and "a mental inhibition", as well as "a cubic object". The ambiguity of "in" in "He ran the mile in five minutes", and "He ran the mile in a paper bag", would also seem to be beyond its powers of resolution.

Third, the blocks world is strongly deductive and logically closed. If gravity were introduced into it, then anything that was pushed in a certain way would logically have to fall. But the common sense world, of ordinary language, is not like that: in the "women and soldiers" example given earlier, the pronoun "several" can be said to be resolved using some generalisation such as "things shot at and hurt tend to fall". There are no logical imperatives there, even though the meaning of the pronoun is perfectly definite. One might summarise these criticisms by paraphrasing a central argument of Wittgenstein's⁶ that the interesting point is not the creation of a language of the blocks world sort, but how different it is from our own.

Indeed, it might be argued that Winograd's system is not about natural language at all, but about how goals and subgoals are to be organised in a problem-solving system capable of manipulating simple physical objects. The definition of "pickup" quoted above is in fact an expression of a procedure for picking up an object in the SHRDLU system. Nothing about it would help one understand the perfectly ordinary sentence "I picked up my bags from the platform and ran for the train."

On the other hand, it has not been shown that the language

facilities just outlined cannot be incorporated in the linguistic structures that SHRDLU manipulates; and even if they could not, the work still would be significant by virtue of its original control structure and its demonstration that real world knowledge can be merged with linguistic knowledge in a working whole.

Paradigms and procedures

Winograd's work is a central example of the "artificial intelligence paradigm of language", using "paradigm" in Kuhn's⁷ sense of a large scale revision in scientific thinking, where the paradigm revised is the generative paradigm of the Chomskyan linguists⁸. From the AI point of view, the generative linguistic work of the last fifteen years has three principal defects. First, the generation of sentences, with whatever attached structures, is not in any interesting sense a demonstration of human understanding, nor is the separation of the well-formed from the ill-formed by such methods; for understanding requires, at the very least, both the generation of sentences as parts of coherent discourse, and some attempt to interpret, rather than merely reject, what seem to be ill-formed utterances. Second, Chomsky distinguishes between performance models, on the one hand, and competence models which are the province of serious linguistic study, on the other. Whatever his intention, this had had the effect of isolating modern generative linguistics from any possible test of the systems of rules it proposes, since testing necessarily involves performance. AI, though it too is concerned with the structure of linguistic processes *per se*, is equally interested in their implementation. Third, there was until recently no place in the generative paradigm for inferences from facts and inductive generalisations, even though very simple examples demonstrate the need for it.

These points would be generally conceded by those who believe there is an AI paradigm of language understanding, but there would be far less agreement over the positive content of the paradigm. The trouble begins with the definition of "understanding" as applied to a computer. At one extreme are those who say the word can only refer to the performance of a machine: to its ability say, to sustain some form of dialogue long enough and sensibly enough for a human interrogator to be unsure whether what he is conversing with is a machine or not. The majority, however, probably argue that this is not enough: the methods and representations of knowledge by which the performance is achieved must be of the right formal sort, and mere performance based on *ad hoc* methods does not demonstrate understanding.

This issue is closely related to that of the role of deduction in understanding natural language, simply because deduction is often what is meant by "right methods". The dispute over deductive methods on the one hand, and other inferential systems closer to common sense reasoning on the other, is in many ways a pseudo-issue because it is so difficult to define clearly what a non-deductive system is: almost any set of formal procedures can be expressed deductively. The heart of the matter concerns the most appropriate form of representation of knowledge, rather than how that knowledge could conceivably be expressed, and it may well turn out that the most appropriate form is nondeductive. The same insight has largely defused another heated issue; whether the appropriate representations should be procedures, as in Winograd's work, or declarations. One disadvantage of procedural representations is that if each item of "knowledge" is attached to a procedure for achieving a particular goal, it will have to be stored separately for each different goal for which it may be used; and if you want to change it later, it will have to be changed separately for each. Another is that conventional, declarative grammars are easier to understand; and Winograd's procedural grammar has been rewritten in more conventional form⁹ for that reason.

While there is now general agreement that any system should show how it is actually to be applied to language, it is no longer fashionable to demand that it must be written in a procedural language, like PLANNER.

"Second generation systems"

When Winograd contrasted his own with what he called second generation systems, he meant, as always in this subject, generations of fashion, not chronology or inheritance of ideas. The foundations and terminology of the approaches he discusses were set out in print in 1966, 1967 and 1968 (refs 10-12). In an already very influential recent paper (unpublished but available from the Artificial Intelligence Laboratory, MIT), Minsky has drawn together strands in the work of these authors and of Charniak¹³ using a terminology of "frames":

"A frame is a data-structure for representing a stereotype situation, like a certain kind of living room, or going to a children's birthday party. Attached to each frame are several kinds of information. Some of this is information about how to use the frame. Some is about what one can expect to happen next. Some is about what to do if these expectations are not confirmed."

"We can think of a frame as a network of nodes and relations. The top levels of a frame are fixed and represent things that are always true about the supposed situation. The lower levels have many terminals—"slots"—that must be filled by specific instances or data. Each terminal can specify conditions its assignments must meet . . . Simple conditions are specified by markers that might require a terminal assignment to be a person, an object of sufficient value etc . . ."

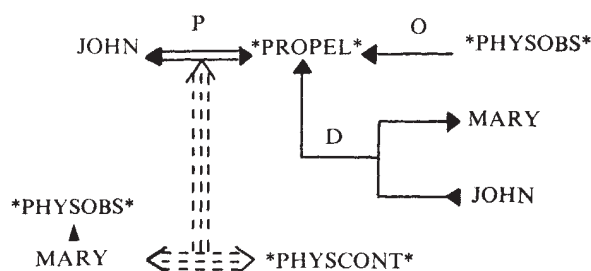
The key point about such structures is that they attempt to specify in advance what is going to be said, and how the world encountered is going to be structured.

In psychological and visual terms, frame approaches envisage an understander as at least as much a looker as a seer. The new work which owes most to Minsky's advocacy is Charniak's. He studied what sorts of inferential information would be needed to resolve pronoun ambiguities in children's stories, and in that sense to understand them. One of his example "stories" is: "Jack was invited to Jane's birthday party. She wondered if he would like a kite. A friend told Jane that Jack already had a kite, and that he would make her take it back".

The problem is to decide whether the penultimate word "it" refers to the first kite mentioned or the second. Charniak's system does not actually run as a program, but was a theoretical structure of rules called "demons" that correspond roughly to what Minsky later called frames. A demon for this example would be "If we see that a person might not like a present X, then look for X being returned to the store where it was bought".

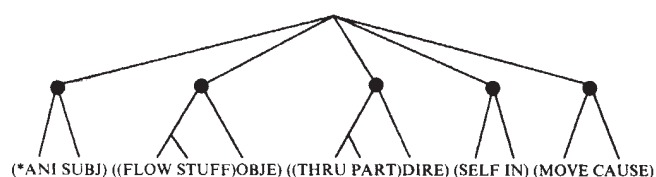
The other systems within the frames approach are rather different. Instead of resting on representations that are superficial in the sense, in which the demon above is just a sentence in English (or in some item-to-item correspondence to one), they are based on reduction to a system of linguistic primitives, and it is at that level that the understander's computations must take place.

Schank's system takes English sentences and produces paraphrase style inferences from them. So, from "John strangled Mary", it produces "John caused Mary to die", "John placed his fingers about Mary's throat" and so on. His internal structures, that he calls conceptual dependencies, may be exemplified by the following for "John hit Mary".



The items between asterisks are primitive actions, and the different types of arrow denote different types of relation.

My own system, which I call preference semantics, runs within an English-French machine translation system, which provides a very clear test of the correctness of the inferences. For example, from "The monkeys ate the bananas although they were not ripe, because they were very hungry" it would translate the two "they's" correctly by different French pronouns because of what it knows about hunger and ripeness. It does this by looking for the most coherent representation in which concepts find their "preferred connections" to others. In this case "ripeness" prefers to be of plants though the system would not balk at "The old man was ripe with years" where that preference cannot be satisfied. It will also tackle examples like the "women and soldiers" one, where it has to bring to bear rules of partial inference about the usual course of the world. These inference rules pattern-match and chain together complex structures of primitives called templates: networks whose nodes have tree structure like the following one for the action "drink":



(*ANI SUBJ)((FLOW STUFF)OBJE)((THRU PART)DIRE)(SELF IN)(MOVE CAUSE)

This is to be interpreted, from the left, as drinking is a causing to be of a preferred liquid object (FLOW STUFF), by a preferred agent that is animate *ANI, and of causing the liquid to be inside the animate thing (SELF IN), via an aperture (THRU PART). The important difference between this system and Schank's on the one hand, and Winograd's on the other, lies not in the semantic markers used, but in the ways they are related in advance in the semantic structures. So, for example, the understander could infer from the above tree structure for "drink", when incorporated in an appropriate template, that, after the act of drinking, the liquid (whatever it is) is inside the drinker. Such an inference would be essential to the resolution of the pronoun in a sentence like "John drank no gin in his cocktail but it felt warm in his stomach nonetheless".

Outstanding points of contention

There are many points of dispute between the proponents of second generation systems: such as whether one should make massive forward inferences as one goes through a text, keeping all one's expectations intact, as Charniak and Schank hold, or whether, as I hold, one should adopt some "laziness hypothesis" about understanding, and generate deeper inferences only when the system is unable to solve, say, a referential problem by more superficial methods.

But the most important argument concerns the question of level: whether the representation should be superficial and highly particular, or deeper and more general. Schank and I hold that the core of understanding consists of very general, and sometimes almost vacuous, inferences about human behaviour, desires and expectations, manipulation of which requires a highly structured primitive representation, and not just the English-words-plus-variables approach of Charniak. This becomes very clear when one actually builds a system that takes English input and deals with it from scratch. Charniak and Minsky believe that this initial "parsing" can be effectively decoupled from the interesting inferential work and simply assumed. But that is not so, because the initial parsing actually depends on many of the later inferences, so the assumption of decoupling can lead to something like a circularity. For example, in analysing "He shot her with a colt", we cannot ascribe any

structure at all until we can make the inference that guns rather than horses are instruments for shooting.

Another distinction arises on the question of syntax analysis. Syntax, which is the heart of Winograd's system, is discounted by both sorts of frame approach, though for very different reasons. Charniak discards it because it is part of the initial parsing from which his inferential work has been "decoupled"; Schank and I do so because we believe semantic analysis to be fundamental, and that in an actual implementation the results of syntactic analysis can all be achieved by a sufficiently powerful semantic analyser. And this last assumption is confirmed by the limited degree of success that the two semantic analysers have actually achieved.

Closely connected with the dispute over the appropriate level of representation of knowledge is another as to what is the most pressing task in the immediate future. I would maintain strongly that work on understanding systems needs a well defined task somewhere between the trivial and the fantastic. If first generation systems sometimes seem trivial, then some of the goals of the more recent work seem fantastic, and at the present stage of our ignorance I would include in that category analysis of any prose, whether children's stories or whatever, that appears to contain problems for the average reader. I find I have to think about the right analysis of Charniak's kite story, discussed above. This is not true for most sentences, even though it is genuinely difficult to specify uniform rules for their analysis. I consider concentration on problem examples, to the exclusion of more natural language, to be a bad inheritance from AI's problem-solving past. On the other hand, not all understanding can be tackled by deep semantic representations: any machine understander that analysed the sense of "good morning" in order to find the correct reply would be making a fundamental error.

Again, a reasonably fluent speaker of, say, German, might well not understand a German conversation about birthday presents without detailed factual information about how Germans organise the giving of presents. Conversely, an expert might understand much of a technical article even with very little

knowledge of the language in which it was written. These considerations tell for Charniak's approach, and it is perhaps paradoxical that the sort of natural language understander that would tend to confirm his assumptions would be one concerned with discourse about, say, the details of repairing a motor car, where factual information is what is central, yet he has concentrated on something as general as children's stories, which need deeper assumptions about human desires and behaviour. That has sometimes seemed to move his research towards the fantastic, and has so far prevented its actual implementation.

In the end the differences about level of representation may again turn out to be ones of emphasis and of what is most appropriate to different subject areas. We should soon see whether that is the case, or whether one "frames approach" is right and the other wrong. It would be nice if this were to be settled by computation rather than by another change of fashion.

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Where is experimental research on earth strain?

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Progress in experimental earth-strain research depends crucially on the measurement capability of its instruments, which are here subjected to critical review.

SERIOUS high sensitivity measurement of earth strain began forty years ago and many interesting experiments have since been proposed. A large number of research themes still await study, however, because of the lack of suitable instruments and installation experience. This article provides an overview of the experimental aspects, including rival mechanical and optical designs, in order to compare their shortcomings and provide us with guides for future research.

What is needed is a measurement capability like that provided by magnetometers or inertial seismometers which can measure a large number of parameters within hours of arrival at virtually any site.

Information to be desired

Depth of penetration of a radiation wave into the Earth increases with signal period. Gravitational potential is, therefore, valuable in investigations of the Earth's deep structure because it provides well isolated forcing frequencies ranging in period from hours to years. It is now virtually routine to extract the frequency spectrum from the original

time domain strain record and numerical examination of this can yield data about the composition of the crust and mantle, and about earth resonances.

Complete specification of regional strain induced in rock requires knowledge of six strain components. If the directions of the principal axes of strain are known the monitoring task can be simplified because fewer components need measuring. At present, neither applied theory or instrument siting procedures are sufficiently advanced for making accurate assumptions about the axes. Indeed, recent reports, concerned with unexpected results obtained with tidal tiltmeters^{1,2} and strainmeters³, indicate that an instrument sees a regional component that can be much modified by the local structure and measurement cavity shape.

If experimental measurements lead us to generalised rules about the direction of the principal axes (criteria which hopefully need no measurements at each site) this should make it possible to investigate regional effects with less than six strainmeter components. Until more is known about this problem it hard to see how isolated strainmeters (singly, in colinear arrays or otherwise) can be used to study regional phenomena.

Theoretical studies will certainly help but multi-com-

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ponent tensor arrays of strainmeters must be used to establish the components at each point: large area strain should not be extrapolated for data from a single strainmeter.

A closely related question is that of strainmeter length. No evidence so far presented proves which interval is optimum. Earlier reports favoured long intervals to average out varying rock properties, but comparative records⁴ now seem to show that length is less critical than was thought. If metre-length gauges could be used it would considerably ease site selection. The first reports of satisfactory short mechanical instruments are published in this volume^{4,5}. Although the feasibility of surface installations^{6,7} has been clearly demonstrated with laser-based instruments the sophistications used contrast sharply with a simple mechanical instrument installed in an underground chamber. It is doubtful if theory alone will be able to provide a close enough model of real local-rock situations for strains are induced by many varied processes. Tensor arrays of cheap instruments could well provide the sought-after generalisations for considerably less effort.

Allied questions are: does regional (fractured, inhomogeneous in practice) rock deform in a truly elastic manner? Are there significant mechanical hysteretic effects? How does fractured hard rock respond? King⁸ has suggested that soft rock locations are preferable but this has not been verified in practice.

Few positive statements can be made about such problems: some answers are beginning to emerge. Mounting methods, for example, show⁹ that the technique used to couple the instrument to the rock is of little importance once an adequate settling time has passed. An instrument can now be installed within the hour to provide useable strain data within days, which represents a considerable improvement over methods of a decade ago.

Before regional effects can be confidently investigated experience must be gained in setting up an instrument consistently. As earth strains do not repeat with time it is of little value to reinstall an instrument to check validity. Instead the simultaneous comparison of two or more instruments on a common Earth interval or a special purpose test base must suffice. Until quite recently this powerful metrological procedure was totally ignored.

Reports of comparisons between instruments are rare. Is this because few attempts have been made or because few have been successful? The seven day runs of the first Invar-catenary instrument and a laser interferometer compared well¹⁰ for most (but not all) of the period. Vali *et al.* published¹¹ the result of a 36-h run obtained with an interferometer and a quartz-tube instrument, but they contain major discrepancies. It is only in the last year that reasonable multiple recordings on a common rock interval have been reported^{4,12}. In both instances the duration of records was long enough to provide confidence in routine use of strainmeters.

Dipping plates at continental boundaries should be subject to abnormal strains. Strainmeters for studying this would need to be very stable and reliable. Any measurement proposals for testing the theory of an expanding Earth^{13,14} fall in this category.

The rate of dissipation of tidal energy has been calculated at about 10^{19} erg s⁻¹. As the resonance quality factor of the solid earth is several orders of magnitude larger than for the oceans, only a small percentage of this energy is dissipated in the solid-tide vibrations. Study of solid tidal signal phase variations might yield an appreciation of the magnitude and uniformity of the energy loss process. Indirect progress toward solid tidal measurements has been made¹⁵ through a better understanding of the ocean loading effects that may mask the expected small phase delays.

There are many other problems. How deep do surface thermal strains really propagate? How important is the adiabatic heating (induced by ambient pressure changes) on

the instrument and the measured rock interval? How does local topography related to the directions of the principal strain axes? How far can the individual strainmeters of a tensor array be separated if a regionally representative tensor is to be obtained?

Are strainmeters good enough yet?

To look at the current state of nanostrain measurements it is necessary to study the fundamentals of each design ignoring the sophistications that can veil the principles. Strainmeters consist of a length standard that is compared against a rock interval using a micro-displacement detection method. The standard used in geophysical strainmeters may be mechanical or optical.

The first mechanical nanostrain gauge used a steel pipe, 20 m long, as the standard with an electromechanical velocity detector. This work by Benioff, in 1935, provided the first tidal records to justify theoretical predictions of rock tide. By 1950 the relatively poor thermal coefficient material—steel—has been replaced by 50 mm diameter fused-silica tube. Many similar instruments have been commissioned since then¹⁶. The choice of transducer is a matter of personal preference for inductive, capacitance and optical alternatives can each provide the sub-nanometre resolution needed¹⁷.

In 1951 Sassa reported an alternative method using an Invar wire suspended between two pillars¹⁸. A mass hung in the centre moves ten times as much vertically as it does horizontally. There are, however, problems of gain variation with sag change. Today there is little need to incorporate mechanical amplification as electrical transducers are able to provide adequate gain¹⁷. A still simpler wire method¹⁹ consists of an Invar wire hanging in an horizontal catenary. A beam balance provides the necessary tension and monitoring facility. Rotation of the balance, as the rock interval varies, drives the transducer.

This method rapidly gained acceptance^{20,22} and about seventy instruments are now in use as the cheapest and easiest strainmeter design available. The Cambridge group operate the majority of operational catenary instruments¹⁶, having installed them in many different global locations.

The chief difficulty in the original catenary design was the relatively large creep in length of the Invar^{21,23} for this required frequent readjustment to maintain the record on-scale. Automatic chart ranging was constructed by two groups^{20,24} to overcome this. (Drift from this cause reduces the precision of secular earth drift estimates and other long-period phenomena.) In many ways²² Invar is not the best material for this purpose. An improved form of tensioned-standard meter has been developed with fused-silica canes linked together as a chain^{12,25}. It was found to exhibit considerably less creep than Invar, improved dynamic performance and better tolerance to thermal contamination. It is important to note that no mechanical strainmeter has been provided with environmental control measures always used in laser instruments.

An interferometer can be used to detect small movements between a distant reflector and a beam splitter or reference reflector of Michelson and Fabry-Perot forms. Claims that this is the almost perfect strainmeter cannot be accepted. Whichever design is used three requirements must be met. First, the radiation must be stable in wavelength to the same order as the drift required (10^{-10} h⁻¹ at least). This calls for the use of the new molecular absorption stabilised lasers. One of the four operational laser designs uses absorption in methane^{26,27}, another in iodine²⁸; one uses a mechanical etalon cavity^{6,7}, a second iodine stabilised unit will be operational shortly²⁹. On this factor alone it is hard to envisage cost reductions reaching a point comparable with mechanical designs. Reliability is also a parameter to be reckoned with at present.

The second necessity is to stabilise the radiation path

because ambient pressure, humidity and temperature variations alter the refractive index (and hence apparent strain) to a degree similar to the effects of environment on a fused-silica standard. An adequate environment is generally provided for optical meters by evacuating the optical path—an expensive investment that is costly to maintain.

The third requirement is to use the interferometer principle for making a transverse comparison with the mechanical rock interval. This involves the use of mechanical components—mounts, optical elements and holders for same. As these are within the measurement loop they may introduce creep and environmental errors which are not easily quantified. One study of Fabry-Perot etalon stability indicated³⁰ that the reflecting film changes were producing cavity length changes of 10^{-8} per month.

Other claims must be carefully considered—that of wide bandwidth, for instance. Counters can respond to 10 MHz with ease but fringe-counting alone rarely provides adequate resolution (1 km interval just suffices). Opto-mechanico interpolation can divide the fringe to 1% but introduces bandwidth limiting mechanisms. The problem is overcome by using methods that provide a beat frequency output but, even so, the frequency stabilisation servos involve the use of bandwidth limiting mirror drivers. Further, the mechanical mounting elements possess natural resonances around 1 kHz so it is unrealistic to talk of extremely wide bandwidth. Furthermore, wide bandwidth is a hollow criteria for crustal movement research rarely needs better than one second response time.

With so few laser instruments running^{7,27,28} it is perhaps still too early for an assessment, but they seem to be inferior to mechanical devices. Several reports include statements about secular strain change: 10^{-10} per day²⁶ and 10^{-9} ground compression⁶. These statements are of limited value for observed drift rates cannot be attributed to ground or instrument as separate entities. (Similar overall drift performance has been achieved¹² with fused-silica instruments.)

Ultimate stability—mechanical or optical?

A mechanical length standard is generally regarded as inferior to an optical one. Recent research, however, has shown that fused-silica can achieve a similar drift rate as the laser method showing that both need consideration in earth strain applications. Brownian movement of the internal molecules of a mechanical object provides a theoretical limit to its length stability. Relative size changes due to this would be around 10^{-15} (one second averaging time) and experimentalists have reached this limit in small devices. It is yet to be shown that a relatively long mechanical bar can be made as stable. The relative wavelength instability of laser radiation can be ascertained from theoretical considerations; the limit is around 10^{-14} for a 1-s integration time. As with mechanical standards this has yet to be reached; practice is currently limited to 10^{-12} instability.

It seems, therefore, that there is little to choose between the two for stability, experimental art being the deciding factor at present.

The ultimate strainmeter?

Mechanical instruments are cheap but prone to environmental effects. A moderate increase in cost could provide the controlled surround which all laser devices have. Experience gained²⁵ with test-base temperature control suggests that microKelvin stability is achievable and that the cost and reliability would be comparable with the vacuum system of a laser strainmeter. Passive³¹, rather than active water circulation, control would further reduce the cost.

Another course of action could be to use ultra-low expansion materials for the standard ($3 \times 10^{-8} \text{ K}^{-1}$ thermal expansion coefficient).

A third, already used, alternative is to monitor the

influencing parameters, correcting the data or physical standard accordingly. This method does not protect the standard from stress cycling—a condition that introduces mechanical hysteresis strain changes. Furthermore it is hard to ensure that the correct parameter is monitored.

These above approaches seem logical but, in fact, do not meet the basic needs of the measurement problem. The current unstated aim is to devise a zero temperature-coefficient instrument placing it in existing underground apertures. On two counts this is incorrect procedure.

First, as the chamber must be relatively large it will modify the parameter sought. Second, long term thermal changes to the chamber will also alter the rock dimensions introducing contaminating components in the record. Ultimately it will become necessary to correct for this.

It seems more logical to opt for an instrument that is submerged in the rock in an insignificant size aperture and which has its overall temperature-coefficient matched to the host rock. It has recently been shown that 1 m fused-silica and Invar strainmeters are feasible so these could be installed into deeply drilled core holes arranged as a shell-burst to provide six components at one place. As the length-standard would always be at the same temperature as the rock, the thermal noise should be reduced without recourse to control of its environment.

Only when it is firmly established what strainmeters measure and when we are able to place them to measure what we need will we be able to significantly advance our knowledge of the deeper Earth. Earth strain measurements are, at last, beginning to be meaningful now that the basic requirements of the problem have been recognised.

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articles

Deep drilling in an active geothermal area in the Azores

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A deep borehole on São Miguel encountered temperatures exceeding 200° C at a depth of 550 m. Subaerial volcanics persist to a depth of 786 m below sea level and indicate an average subsidence of 0.1 cm yr⁻¹ for the island over the past 690,000 Myr.

DEEP drilling is essential to the understanding of the process of the formation of oceanic islands. The petrology and geochemistry¹ of island rocks differs significantly from those of deep ocean crustal rocks and the structure underlying islands as defined by geophysical data differs from that beneath deep ocean floors. The contrast suggests that special deep processes operate beneath oceanic islands. Most volcanic islands are confined to near spreading plate margins during their active period and their formation may be associated with the ocean floor spreading process^{2,3}. Periodically, voluminous volcanic activity indicates exceptionally high rates of subcrustal convective heat transfer and mantle hot spots or plumes have been postulated as the sources of this activity⁴.

In 1972 geoscientists from Dalhousie University and Lamont-Doherty Geological Observatory initiated a deep drilling programme into the oceanic crust with an 800 m hole into the island of Bermuda in the western Atlantic⁵⁻⁷. It yielded some 1,000 volcanic units approximately equally divided between thin altered tholeiitic pillow lavas and lamprophyric intrusive sheets. Our second borehole in 1973 was located on the island of São Miguel, in the Azores, and an initial report is presented here of the 981 m penetration of a complex sequence of subaerial and submarine lavas, pyroclastics and volcanogenic sediments.

The Azores form a group of nine islands aligned in a NW-SE trending chain which transects the Mid-Atlantic Ridge (MAR) at 39° N (Fig. 1). The East Azores Fracture Zone and the MAR intersect at a triple junction⁸ in the vicinity of the Azores and the region is marked with (i) a sharp change of trend of the MAR, (ii) the broadening of the ridge into an extensive platform⁹ (Fig. 1) and (iii) an unusually high positive regional gravity anomaly¹⁰. The islands east of the MAR trend obliquely to the proposed plate margins and follow a pronounced bathymetric lineament (Fig. 1), the Terceira Rift^{9,11} which extends through

parts of Graciosa, Terceira and São Miguel. Krause and Watkins⁹ suggest that the Terceira Rift represents a secondary spreading centre trending towards Gibraltar which originated approximately 45 Myr ago.

São Miguel, the largest of the islands, lies near the eastern extremity of the chain 400 km from the MAR crest. Its surface geology is dominated by four large calderas, three of which have erupted during historic times¹² and have produced an extensive blanket of trachytic pumice which covers much of the island^{13,14}. Surface rocks range compositionally from alkali basalts to trachytes^{15,16} and are exceptionally potassic¹⁷. We selected a drill site on the gentle flanks of the volcano Agua de Pau which rises to a maximum height of 950 m above sea level and has a diameter at sea level of approximately 15 km. Its surface deposits consist of voluminous trachytic pumice and associated basaltic lavas intercalated with occasional trachytic extrusives. The last recorded eruption from the summit caldera in 1563 AD produced a cover of trachytic pumice and Walker and Croasdale¹⁸ document four similar eruptive events during the past 4,600 years. Agua de Pau must therefore be considered to be currently dormant, as sporadic seismic activity beneath the caldera and a number of boiling hot springs on the flanks also indicate.

Stratigraphy and petrology of the core

Drilling began at 72 m above sea level, but only partial core recovery was possible in the first 148 m because of unconsolidated pyroclastic and mud flow deposits. Below this depth core recovery was virtually continuous to 981 m where drilling terminated following steam production. A synoptic core log is shown in Fig. 2.

Extrusive lavas constitute 72% of the drill core and occur in 140 separate flow units of 4.8 m average thickness and 2.5 m median thickness. Alkali basalts, hawaiites and mugearites predominate and only three trachyte flows (6% of total flow volume) were encountered. In the top 763 m of core a subaerial origin for the flows is suggested by (i) massive nature of flow centres, (ii) complete absence of pillow structures, (iii) intercalation of numerous pyroclastic units lacking any indications of aqueous reworking or stratification, (iv) development of some lateritic horizons, and (v) occurrence of thick vesicular and autobrecciated

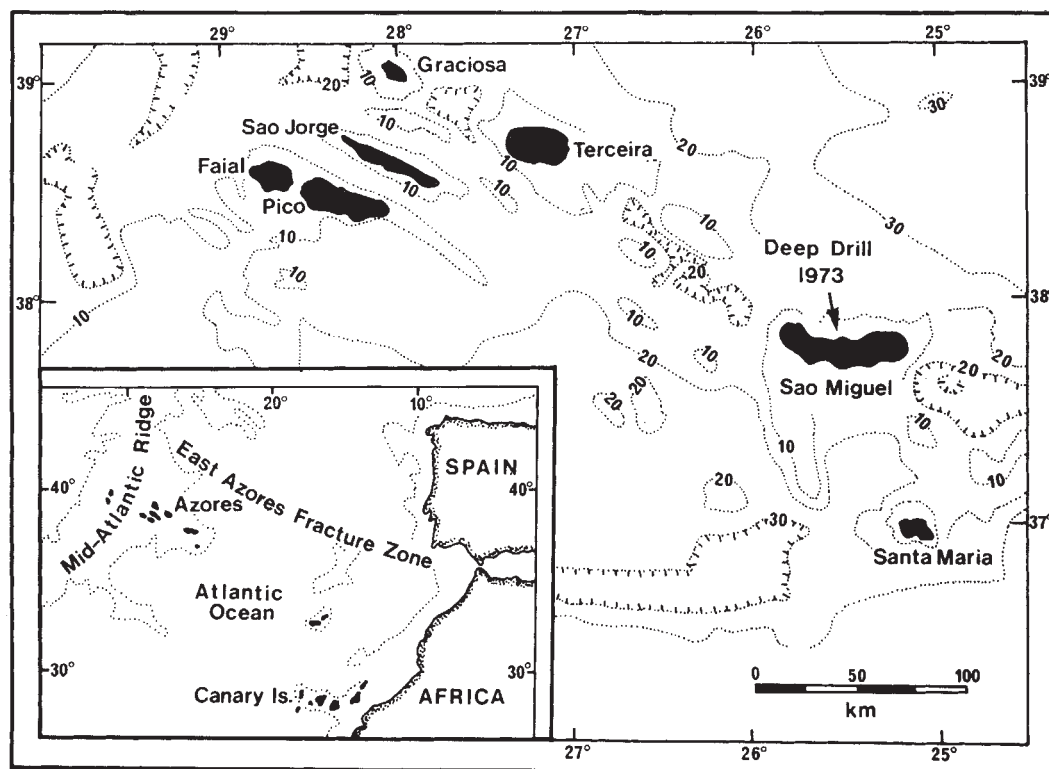


Fig. 1 Location map of Deep Drill 1973. Site coordinates $25^{\circ} 31.4'W$, $37^{\circ} 48.9'N$ at 5 km from the caldera wall of Agua de Pau volcano, São Miguel. 1,000 m bathymetric contours are shown⁸.

flow tops. Pillowed basaltic rocks with altered glassy margins were first encountered at 880 m where they are found interbedded with massive basaltic flows in a sequence devoid of pyroclastics.

In sharp contrast to the Bermuda drill core³, intrusives constitute an insignificant fraction in São Miguel. Near vertical basaltic porphyry sheets in chilled contact with basaltic flows were encountered only at 662 m and 738 m.

Pyroclastic deposits, chiefly trachytic in character, account for 22% of the core and range from fine-grained tuffs to pumice deposits, agglomerates and mud flows. Indications of bedding are rare but when seen can be inclined steeply up to $30-40^{\circ}$. Steep depositional dips can also be seen in the recent ash-pumice deposits but these may not be an indication of tectonic tilting since contacts between flow units appeared to be near horizontal over the entire length of the core. Ignimbrite cooling units ranging in thickness from 6 cm to 3.8 m are common in the depth interval 262 m to 508 m. Only two such units have previously been described on the island¹⁷. Individual pyroclastic units are frequently trachytic in character at the base but grade upward into more basic compositions.

A 107 m thick igneous-sedimentary sequence marks the transition from subaerial to submarine volcanics. Basaltic breccias with a chloritic matrix (altered glass? or ash) are interbedded with massive flows in the upper part of the sequence (Fig. 3) and overlie 15 m of bedded coarse lithic sandstones. One 7 m bed consisting of angular basaltic fragments embedded in a matrix of black lithic sand (carbonate-quartz cemented) is strikingly similar to the black beach sands being formed along the Hawaiian coast line by flows entering the sea (Tilling, personal communication, 1974).

Temperatures

Bottom hole temperatures were monitored during drilling and temperature logs were measured at various intervals after drilling ceased (Fig. 4). The temperatures show $200^{\circ}C$ water progressing upward from 550 m at the start to 290 m at the time of the last measurements. The boiling point was exceeded and steam erupted from the hole when the drill

rod was removed. The $200^{\circ}C$ water would have boiled first when it reached the temperature-pressure boiling curve near 215 m depth. The steps in the last temperature profiles (for example at 110 m, 170 m, and 305 m) were produced by the convection water circulation in the hole because of loose ash sequences in which drill rods enlarged the hole considerably. Convection circulation in the hole is limited upward by tight basaltic and trachytic flows. It was also restricted above 305 m by casing.

Temperatures were nearly constant at 20° to $25^{\circ}C$ to 100 m depth, a sudden jump to over $100^{\circ}C$ occurred between 100 m and 175 m, then a uniform gradient of about $250^{\circ}C\ km^{-1}$ to a depth of 550 m and finally a very small temperature gradient of less than $10^{\circ}C\ km^{-1}$ to the bottom of the hole (Fig. 4).

In general, temperature profiles in hydrothermal areas show low vertical gradients in permeable rocks where the geothermal heat is carried upward by convection¹⁸. Impermeable rocks show high gradients since the heat must be carried through them by conduction. The region of this borehole is clearly not a normal hydrothermal system since the generally high porosity (and presumably high permeability) subaerial section above 550 m has a high gradient while the less permeable rocks below 550 m have a very low gradient. We suggest the following model to explain the observed temperatures.

The complete section penetrated by the hole probably does not permit vertical convection. Even though there are extensive permeable rocks (pyroclastics) above 550 m, circulation is restricted by frequent horizontal, impermeable horizons (flows). The lack of cellular convection is seen by the rapid change in measured temperatures with time when convection became possible in the borehole itself. Water flow parallel to the bedding (nearly horizontal), however, is probably not restricted in the permeable pyroclastic horizons. We suggest that there is hot water ($205^{\circ}C$) flowing parallel to the bedding at about 550 m and water at $100^{\circ}C$ flowing just under the impermeable layer at 110–120 m depth. The hot water probably moves down-dip in these horizons from its source in the upper regions of the volcano. There is no clear evidence for water flow in other

horizons. The low temperature gradient below 550 m suggests that there is no volcanic heat source directly beneath the drill site itself.

It is difficult to estimate the amount of hot water available in this area for geothermal power since no flow measurements were made. The hole erupted briefly with hot water and steam but was stopped after only 20 min, before any depletion of flow could be detected. The permeability of the core also has not been measured. Large volumes of 200°–210° C water may be available from near 550 m depth if the down-dip flow in the permeable horizons from higher on the volcano is rapid but flow tests are required to confirm this conclusion.

Age determinations

Palaeomagnetic measurements on lava flows in the core indicate that the subaerial, transition and subaqueous sequences are all normally magnetised. This probably indicates magnetisation during the Brunhes polarity epoch, although the possibility of remagnetisation during hydrothermal alteration cannot be discounted. If magnetisation does reflect geomagnetic field polarity the time scale of reversals of polarity of the Earth's magnetic field²⁰ provides an upper age limit of 0.69 Myr for the formation of the entire volcanic-sedimentary sequence.

Two samples were selected for radiometric dating by the conventional K-Ar method. The first of these, a slightly impure sanidine concentrated from a fresh trachytic flow located at 57 m depth has an apparent age of $(117 \pm 24) \times 10^3$ yr (% $K_2O=6.7$; $^{40}Ar_{radiogenic}/^{40}Ar=0.5$). The second sample, a relatively fresh submarine lava from the 950 m level has an apparent whole rock age of $(280 \pm 140) \times 10^3$ yr (% $K_2O=2.7$; $^{40}Ar_{radiogenic}/^{40}Ar=0.013$). In other words, this

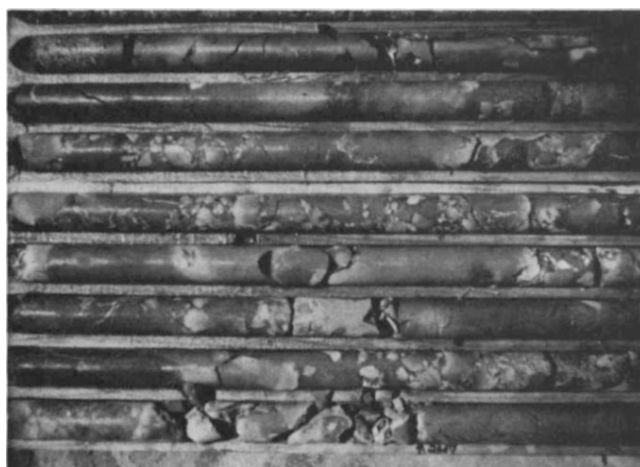


Fig. 3 Breccia from the transition zone consisting of angular basalt fragments of variable size in a matrix of coarse vulcanogenic sand and chloritic material. Basalt fragments show conspicuous leached margins. Length of core segments 0.6 m.

rock appears to have retained at least some radiogenic argon in spite of high ($\sim 205^\circ$ C) temperatures encountered. The sample contained $\sim 2.5 \times 10^{-6}$ s.c.c. g^{-1} atmospheric argon, an unusually high value perhaps indicative of exchange of gases between the lavas and overlying hydrothermal sources. We conclude from the radiometric and the magnetic data that the drill core spans a time interval of the order of a few hundred thousand years in the Brunhes normal polarity epoch and that the major volcanism probably ended about 100,000 years ago.

Deep drilling on São Miguel has disclosed several important aspects of the island's geological evolution which are not evident from an examination of its surface geology. Three distinct subaerial eruptive sequences totalling 762 m in thickness were found to overlie a complex 107 m transition zone which documents the changeover from a subaerial to a submarine environment. Each subaerial eruptive sequence commenced with a quiescent phase of basaltic lava extrusion and was followed by increasingly explosive activity. Intercalated tuff beds increase in frequency and thickness and grade into a terminal phase of trachytic pumice deposition with rare trachyte extrusion. Compositional zonation within individual pyroclastic units further supports strong compositional zonation in the magma chamber at this stage. We suggest that each eruptive sequence signals the arrival of a fresh magma batch from depth in the mantle into a shallow magma chamber. Crystal fractionation and possibly assimilation at shallow depth would then yield increasingly differentiated products and an enrichment in volatiles; a combination which would in each case lead to increasingly explosive acidic eruptive activity.

Island subsidence

A surprising result of our experiment has been the depth at which submarine deposits were encountered. Subaerial or shallow marine conditions are found to 786 m below present sea level and indicate substantial subsidence of the island. Postglacial sea level rise since 18,000 BP can account for only 130 m of subsidence²¹. An evaluation of the glacial eustatic control of sea level for the earlier Pleistocene does not seem possible because of the largely unknown influence of tectonic crustal movements on sea level, but is not likely to be much greater. Paleomagnetic and radiometric dating of the core at younger than 0.69 Myr BP yields a minimum average subsidence rate of 0.1 cm yr^{-1} . Other data on the vertical crustal motion in the

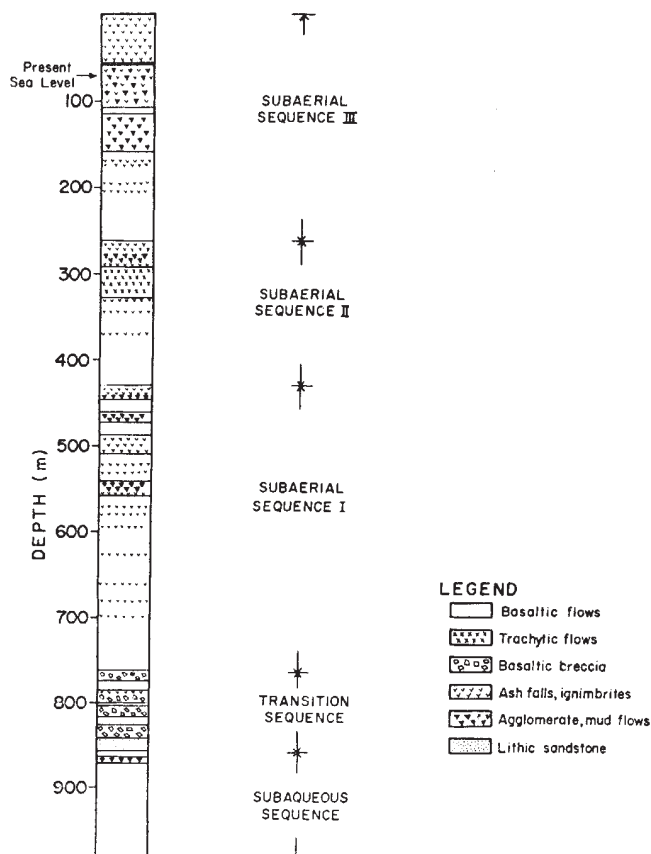


Fig. 2 Log showing major lithologic variations in the São Miguel drill core.

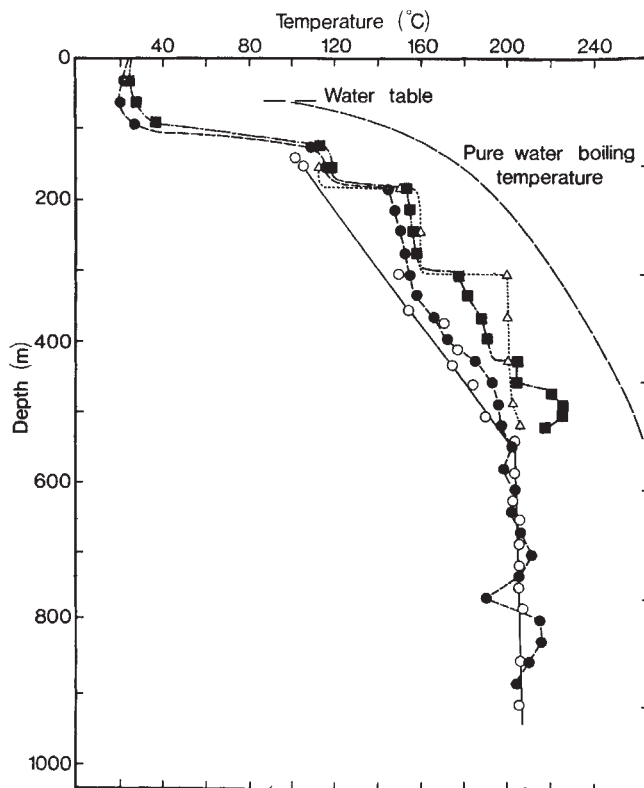


Fig. 4 Temperature measurements with maximum thermometers ($\pm 2^\circ\text{C}$) to 914 m during drilling; to 885 m with thermocouple ($\pm 1^\circ\text{C}$) between 1 and 2 h and again 4 days after termination of water circulation. Two days after further circulation maximum thermometer measurements were made to 518 m. O: temperatures at the bottom of the hole; temperature logs: $\bullet$: 26 August (1–3 h); $\blacksquare$: 2 September (4 d); $\triangle$: 7 September (3 d).

Azores come from São Miguel's closest neighbour, Santa Maria, where a submarine sequence of basaltic volcanics and sediments of Miocene to Quaternary age is exposed²². This implies uplift for the island and suggests that the subsidence of Agua de Pau volcano is a local phenomenon.

The subsidence of ancient oceanic islands to form seamounts has been attributed to the thermal contraction of the lithosphere as it moves away from the spreading centre^{23–25}, or to the vertical motion of the lithosphere as it slides over a bumpy asthenosphere^{26,27}. In either case subsidence rates calculated from known spreading rates or theoretical models yield values of $0.01\text{--}0.02\text{ cm yr}^{-1}$ which is an order of magnitude below those observed on São Miguel.

Detailed information on the vertical motion of other active volcanic islands is scarce. An analysis of tide-gauge measurements in the Hawaiian islands²⁸ reveals recent subsidence rates of 0.5 cm yr^{-1} for Hawaii, 0.2 cm yr^{-1} for Maui, and stable conditions on Oahu. Decreasing subsidence rates correlate with an increase in the geological age of the islands and a decrease in intensity of their recent volcanic activity²⁹. Ward³⁰ has used raised shorelines and drilling data³¹ from Oahu to demonstrate that Pliocene subsidence of the island was followed by uplift of $1.6\text{ cm } 1,000\text{ yr}^{-1}$ during the Pleistocene.

São Miguel and adjacent Santa Maria exhibit a similar pattern. On Santa Maria, which is undergoing or has undergone emergence, neither historic eruptions nor any hot spring or fumarolic activity are reported. Volcanic activity has been dated to extend back 8.2 Myr^{32} . The island is also furthest removed from active spreading centres such as the Terceira Rift. São Miguel, by contrast, shows rapid subsidence, active volcanicity, surface lavas dating from the

historic era back to only 4.0 Myr^{32} , and is situated on the currently active Terceira Rift.

The subsidence of an active volcano like São Miguel arises primarily from crustal loading with the addition of volcanic material. In Hawaii, Swanson³³ believes that simple isostatic adjustment adequately accounts for the observed subsidence. On a simple isostatic model the subsidence of 800 m observed on São Miguel requires the addition of a thickness of about 1 km of subaerial volcanic material to the island and will result in an increase of average elevation of 100–200 m. When a volcanic island becomes extinct, such as Santa Maria, erosion will result in isostatic rebound or uplift. If the top of the submarine section was 800 m below sea level when the island was active, at least 1,000 m has been eroded with a general reduction of elevation of 100–200 m.

Our temperature measurements suggest that the northern flank of Agua de Pau represents a promising prospect for future geothermal exploration. Bottom hole temperatures are well within the range of productive geothermal fields³⁴ and occur at profitable depth. The thick subaerial volcanic sequence with its high proportion of pyroclastics and thick scoriaceous flow tops provides high porosities and possibly also permeabilities which make high flow rates probable. Further investigations to determine total hot water reserves, possible extraction rates, and the chemistry of the steam and water in this and other wells will be necessary for more specific evaluations. Seismic profiling to evaluate trap conditions near our borehole would seem a sensible next step.

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Observation of tissue metabolites using ^{31}P nuclear magnetic resonance

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^{31}P NMR spectra of intact biological tissues can now be observed. The use of the spectra to study the course of reactions within the tissues is illustrated by experiments on muscle and its glycogen particle fraction.

ALTHOUGH phosphorus NMR has only $1/15$ the sensitivity of proton NMR, it is attractive because studies may be done in aqueous solution and the spectra are relatively simple because of the small number of different chemical environments in which phosphorus atoms are found. In addition, the chemical shift range is much larger for phosphorus than for protons.

As a result of considerable instrumental improvements achieved recently in both the magnitude of the magnetic field and the sensitivity of the detection¹ we are now in a position to study ^{31}P resonances in a large variety of systems at concentrations found naturally in biology, using Fourier transform and impulse response techniques². Useful spectra can now be obtained of systems varying in complexity from solutions of purified enzymes to intact tissues.

Phosphate resonances

At the high magnetic field strength employed (7.5 tesla) the ^{31}P resonances of a large number of biologically important phosphate-containing compounds can be resolved. The chemical shifts of the phosphate groups are in the range of about 30 p.p.m., and many sugar phosphates and glycolytic intermediates can be resolved. Furthermore, the state of ionisation of the phosphates and their interaction with metal ions, such as Mg^{2+} , affect the positions of the resonances. Figure 1 shows ^{31}P spectra of a mixture of compounds recorded at various pH values, the individual resonances having been assigned by observing the spectra of the individual components.

The resolution of the resonances from various phosphorus-containing small molecules allows rapid assay of the components of mixtures of these molecules. Measurements are carried out without destruction or dilution of the sample, and provide a method of monitoring turnover and interconversions of these molecules in organelles.

In the glycogen particulate fraction isolated from rabbit muscle, covalent enzyme regulation has been exhaustively studied^{3,4} and transient phosphorylation of phosphorylase *b* has been shown to be the principal trigger for glycogen breakdown. The enzymes concerned are also regulated by small ligands. Therefore, knowledge of concentrations of these regulators at any point in the transient covalent activation is important for full understanding of the control mechanism⁵. Figure 2 shows the turnover of the phosphorus containing

ligands during a typical transient activation, obtained from a series of ^{31}P NMR spectra, recorded on a single sample. The active form of phosphorylase immediately catalyses glycogen breakdown, leading to the production of glucose-1-phosphate and so (by phosphoglucomutase activity) to glucose-6-phosphate. The production of glucose-6-phosphate is concomitant with phosphate utilisation. During the phosphorylation of phosphorylase *b*, ATP is converted to ADP. Because of the presence of adenylate kinase, which catalyses the reaction $2\text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$, and of AMP-deaminase, which catalyses the reaction $\text{AMP} \rightarrow \text{IMP} + \text{NH}_3$, the ADP initially formed is depleted and IMP—not distinguishable from AMP by ^{31}P NMR—is produced. The instability of the nucleotide levels indicates that the system is not a very good model for extrapolation to the *in vivo* situation.

The assay technique outlined above has also been used to estimate the separate activities in a crude extract of homogenised rabbit muscle by following the sequential production of glycolytic intermediates. The mass action ratios for some of the

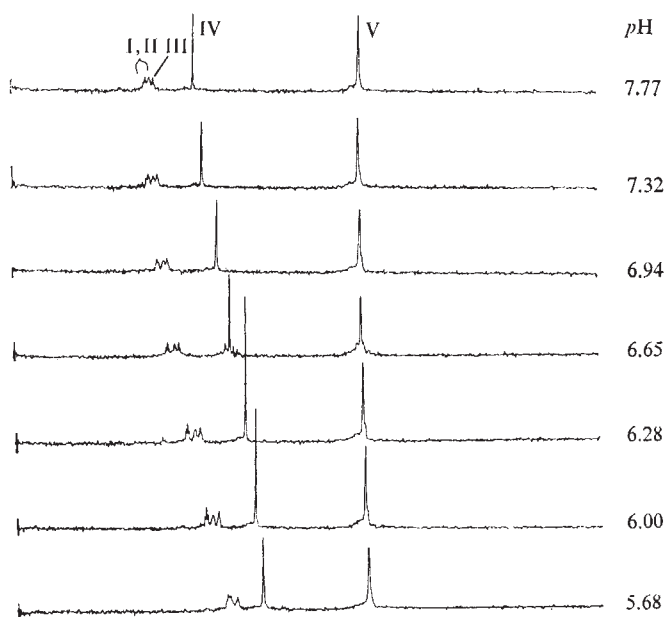


Fig. 1 ^{31}P NMR spectra of a mixture of fructose-1,6-diphosphate (I, II), IMP (III), inorganic phosphate (IV), creatine phosphate (V) at various pH values, recorded at 129 MHz. No buffer present; total phosphate concentration 60 mM. Sweep width 5 kHz, pulse interval 2 s, 200 scans. Spectrum recorded without proton irradiation.

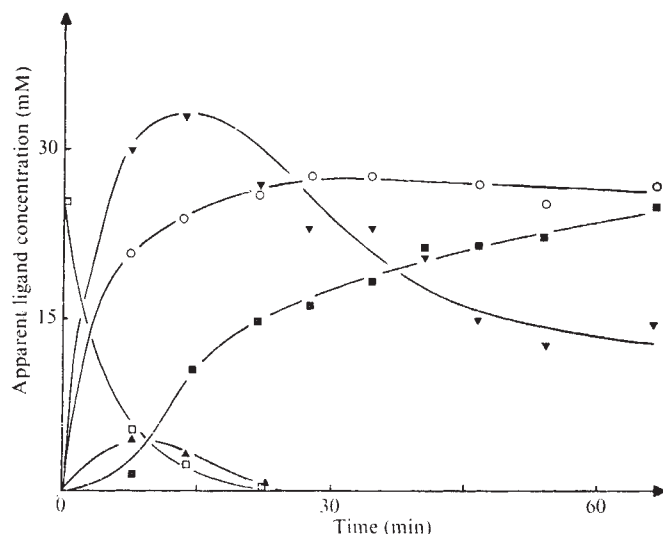


Fig. 2 Variations in concentrations of $\square$, ATP; $\blacktriangle$, ADP; $\circ$, AMP and IMP; $\blacksquare$, glucose-6-phosphate; and ∇ , inorganic phosphate during flash activation of phosphorylase in glycogen particles. (27 mM ATP, 25 mM MgCl_2 , 1 mM CaCl_2 , 50 mM triethanolamine, 100 mM potassium chloride, 1 mM EDTA pH 6.9). ATP was added at time zero. Sweep width 5 kHz, Pulse interval 3 s, 50 scans. Spectrum recorded without proton irradiation. Differential saturation of the resonances can lead to small errors in the relative concentrations of ligands as measured from the areas under the peaks. The measured concentration of any ligand, however, is always a fixed proportion of its true concentration.

individual reactions have been deduced from the results obtained. The technique has also rapidly revealed the presence of contaminant activities in supposedly pure enzymes.

Metabolites in whole muscle

Our attempts to follow enzyme activities in intact tissues using phosphorus NMR have, to date, been principally concerned with observations on muscle from the hind leg of the rat. Figure 3 shows the ^{31}P spectrum of an intact, relaxed muscle, freshly excised from a rat killed by etheration. Assignments are made on the basis of the pH titrations mentioned in section 1. Part of the 'sugar phosphate' peak is attributed to

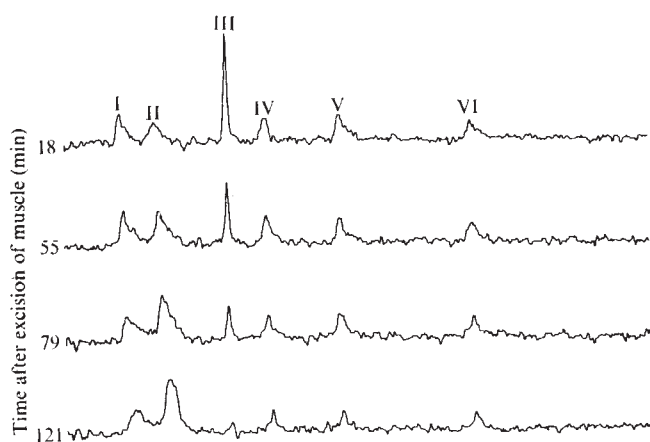


Fig. 3 ^{31}P NMR spectrum of an intact muscle from the hind leg of the rat recorded at 129 MHz, without proton irradiation. Temperature 20°C and pulse interval 16 s. Peak assignments: I, sugar phosphate and phospholipid; II, inorganic phosphate; III, creatine phosphate; IV, γ ATP; V, α ATP; VI, β ATP. The times are the midpoints of the 50 scan spectral accumulations (referred to excision time as zero). The muscle was bathed in a minimum volume of calcium-free Locke ringer.

phospholipid as there is a broad peak of identical chemical shift in the phosphorus spectrum of debris from muscle extracted with aqueous buffer. Assays on this aqueous extract record a concentration of about 1 mM for glucose-6-phosphate. The frequency of the inorganic phosphate resonance defines the apparent pH of its environment (7.1 in this spectrum) and the frequencies of the ATP peaks correspond to those for the Mg^{2+} -ATP complex at the phosphate pH. (The frequency of the creatine phosphate resonance is independent of pH around 7.) *In vitro* studies of Mg^{2+} binding to ATP show that there are large shifts (2–3 p.p.m.) in the β - and γ -phosphate resonances on complex formation. These shifts show that the ATP observed in muscle is almost entirely complexed to magnesium ion. Changes in ionic strength also produce measurable spectral shifts, but these are much too small to alter our assignments for the intact muscle spectrum.

The signal peaks of the muscle phosphates are broader than those for the corresponding compounds free in solution, and the inorganic phosphate peak is markedly broader than the ATP or creatine phosphate resonances. The explanation for this phosphate broadening cannot lie in a disturbance of magnetic field homogeneity caused by the nature of the sample as the width of the creatine phosphate signal gives an upper limit for

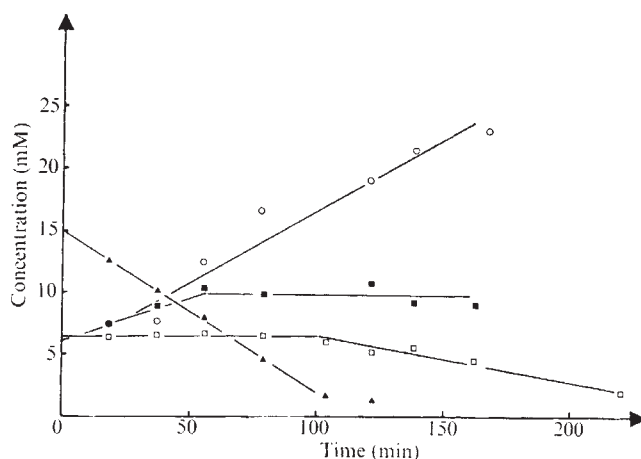


Fig. 4 Variation of phosphorus metabolite levels in an intact rat leg muscle with time after excision. The integrals of spectra shown in Fig. 3 are plotted in this graph. $\circ$, Inorganic phosphate; $\blacktriangle$, creatine phosphate; $\blacksquare$, sugar phosphate and phospholipid; $\square$, ATP. An absolute concentration scale was established by running a standard sample of 10 mM phosphate in the same conditions as the muscle.

the field inhomogeneity. Inorganic phosphate has a pK at 6.9 whereas the nearest pK for creatine phosphate is at 4.6. The frequency of the former resonance is therefore strongly pH dependent around pH 7 whilst that of the latter is pH independent in this region. It seems likely that the large width of the phosphate line may be due to a distribution of pH within the muscle, that is to some partial compartmentation of inorganic phosphate. The pH range that would account for the linewidth is about 0.5 pH units.

We have measured the approximate concentrations ($\pm 10\%$) of phosphorus metabolites in the intact muscle by integration of the absorption spectrum, using a sample of known concentration as a standard after taking care to avoid saturation of the resonances. There is some variation from muscle to muscle, and the creatine phosphate:phosphate ratio proves to be a sensitive index of the degree of stimulation of the rat muscle before the animal's death, that is a high creatine phosphate:phosphate ratio in rats killed without excessive stimulation. We have measured the concentrations of 'sugar phosphate', inorganic phosphate, creatine phosphate and ATP at intervals between 16 and 170 min from excision of the muscle (Fig. 4). Extrapolation along the time axis shows the metabolite levels

at the time the muscle was excised to be: sugar phosphate+phospholipid, 6 mM; inorganic phosphate, 6 mM; creatine phosphate, 15 mM; and ATP, 6.5 mM.

Creatine kinase maintains the ATP level constant at the expense of creatine phosphate until all the latter substrate has been used up, demonstrating the ability of the kinase to buffer the muscle ATP concentration⁶. Ageing of the muscle is accompanied by a fall in pH which is monitored by changes in the frequencies of the phosphate and ATP resonances. The pH at the start of data accumulation is 7.1, and after 160 min it has fallen to 6.2. Acid accumulation seems to accelerate at the time when the creatine phosphate concentration falls to zero and glycolysis presumably begins.

These results can only be obtained with intact muscle. Samples which are even slightly lacerated during handling have only an inorganic phosphate peak in the phosphorus spectrum. Breakdown of organic phosphates by phosphatases is assumed to have occurred in the damaged muscle.

Scope

On the basis of these experiments and other data we have obtained from a variety of biological systems, we believe that ³¹P NMR can yield general information about metabolite levels, turnover, interactions and compartmentation. As it is

also possible to distinguish between signals from extracellular and intercellular materials, in favourable cases the study of certain transport processes is now also possible. The delineation of unusual pathways, the detection of possible new intermediates and the presence of bound nucleotides also seems within the scope of the method. To what extent the kind of structural information that is beginning to emerge from solution studies can be extended to the study of intercellular components remains to be determined.

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letters to nature

Degeneracy effects on neutrino mass ejection in supernovae

THAT type II supernovae apparently occur predominantly in stars significantly more massive than the Sun has been known¹ for some time, although a precise stellar mass range for these cataclysmic events can not be defined observationally. Recent theoretical interpretations^{2–4} of diverse observational data seem, however, to indicate independently that the masses are significantly in excess of $8M_{\odot}$. Furthermore, observational evidence⁵ may exist that stars with masses below $6M_{\odot}$ cease nuclear burning before a critical supernova configuration evolves. If this is the case, the total supernovae contribution from the stars of lower mass is negligible and theoretical difficulties⁶ associated with such events are resolved. The circumstantial evidence for massive ($>8M_{\odot}$) supernova progenitors is thus reinforced, although problems arise in understanding the supernova mechanism.

Attempts to explain supernovae in massive stars theoretically have centred on either nuclear fuel detonation⁷ or neutrino energy deposition⁸ in the infalling envelope during dynamic stellar collapse. The former mechanism does not release sufficient energy (ref. 8 and Z. K. Barkat, J. C. Wheeler, J. R. Buchler and G. Rakavy, unpublished) to account for the type II explosion. The published results⁹ on the latter are in dispute^{10,11} but tend to be negative with respect to the required mass ejection. Recently it was suggested¹² that the neutrino-matter coupling may be enhanced considerably through coherent scattering from nuclei. Subsequent hydrodynamic computations¹³ required a fivefold increase in the theoretically estimated cross section for effective neutrino mass ejection. As coherent effects diminish at the high average escaping

neutrino energy⁹ (~ 50 MeV), the results are not very encouraging. Thus the posited mechanisms for massive stellar explosions seem inadequate.

The strong circumstantial evidence for massive supernovae progenitors, however, requires a critical re-evaluation. The comments in this communication will focus on the neutrino transport mechanism. All extant computations impose the condition of a zero chemical potential on the neutrinos. Arbitrary variations in the neutrino numbers result as a photon-like blackbody spectrum is maintained. Neutrinos and antineutrinos contribute to the total lepton number, however, and therefore can not be created or destroyed arbitrarily. Indeed I shall argue that the arbitrary zero chemical potential condition suppresses leptonic neutrino transport characteristics that may lead to supernova mass ejection.

My study was limited to high densities ($>10^{11}$ g cm⁻³) where the diffusion approximation to neutrino transport obtains¹¹. In this approximation the Boltzmann transport equation transforms by way of the Lorentz-Enskog method into a relationship between the nonisotropic neutrino intensity and the gradients in temperature and chemical potential. Appropriate integration over the neutrino spectrum (the canonical electron scattering opacity¹⁴ was assumed) then yields the Rosseland mean approximations for two neutrino fluxes, one for energy and one for particle number¹⁵.

Comparison of the resulting coupled energy/particle diffusion equations with that of the usual diffusion approximation¹⁶ indicates that the latter suppresses two potentially significant leptonic effects: energy transport caused by chemical potential gradients, and variations in the efficiency of thermal transport with particle number concentration (that is, chemical potential variations). As they have not been accounted for in the extant computations, the question arises of their significance for neutrino supernovae.

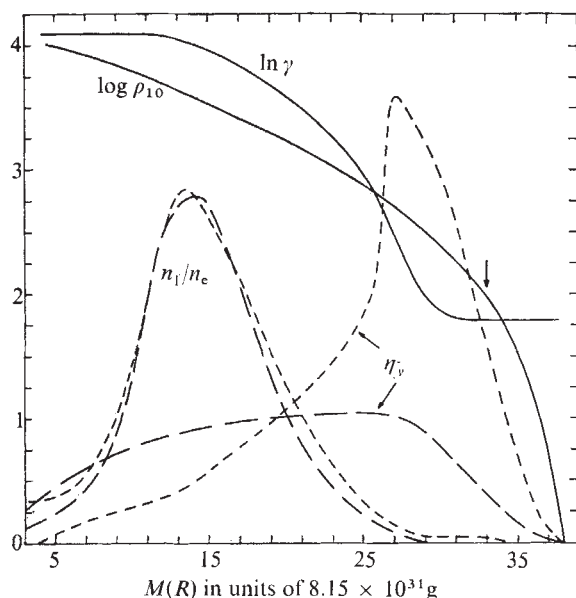


Fig. 1 The distribution of diffusion parameters with the total mass interior to a given radial point in the collapsing structure after diffusion has progressed for 0.445 ms. The parameters shown include the temperature ($kT = \gamma m_e c^2$), the density ($\rho = 10^{10} \rho_{10}$), the ratio of the chemical potential to kT (η_v), and the ratio of the neutrino lepton number to that of equilibrium matter electrons. The arrow indicates where the gravitational free-fall time scale equals the elapsed time. —, Coupled; ----, decoupled.

For a quantitative answer, the degree of neutrino particle/antiparticle interaction must be determined. Rapid interaction on transport time scales gives neutrino/photon equilibrium and will be referred to as the coupled transport mode. When no interaction is possible the decoupled mode obtains. Coupling may be mediated either through particle/antiparticle annihilation or through equilibrium in both urca reactions:

$$\nu_e [(Z, A), (Z+1, A)] e^- \text{ and } \bar{\nu}_e [(Z+1, A), (Z, A)] e^+.$$

Electron pair production should dominate in neutrino annihilation as one and two photon processes cannot contribute¹⁷. Hence coupling will decrease with electron degeneracy. For thermalised neutrinos, coupling may be impossible on energetic grounds alone in electron-degenerate outer regions of collapsing supernova configurations, and a transition from coupled to decoupled transport may be possible between the hot central condensation and the collapsing outer envelope. The details of such a transition were ignored in this preliminary study, and only the extremes of total coupling and decoupling were investigated.

Neutrino diffusion was studied in a supernova ambience to determine the leptonic effects in transport. Coherent scattering by nuclei was neglected; thus muon neutrinos escaped essentially without interacting. The investigation used a stationary collapsed stellar density structure that corresponds to published results⁹ of hydrodynamic collapse at a given instant. The initial temperature structure was essentially that of Fig. 1 and the chemical potential was initially set to zero throughout. The shock energy and lepton number deposition was approximated by means of source terms using published shock structure estimates¹¹. Ninety per cent of this energy was assumed to escape in the form of muon neutrinos⁹. The electron neutrino lepton number increased at the rate of matter transport through the shock to higher density with lower electron number¹⁸. The two equations for diffusion of energy and of particles were solved numerically for the coupled and decoupled transport modes. Fluxes were allowed to escape without interaction for densities below $10^{11} \text{ g cm}^{-3}$.

The resulting configuration after 0.445 ms of evolution is shown in Fig. 1. A striking result for both transport modes is the concentration of neutrino lepton number at the shock front (its value was initially zero). The arrow indicates the point at which the hydrodynamic free-fall time equals the elapsed time of diffusion. The hydrodynamic time scale rapidly diminishes at the higher densities at which the neutrino lepton number concentrations occur. The neutrinos are therefore virtually trapped on hydrodynamic time scales and will fall with the matter. The rate of neutrino lepton number generation at the shock front is therefore greater than that assumed in the present static investigation. The actual hydrodynamic collapse of the matter will aggravate the chemical potential build-ups obtained.

The distributions of chemical potential for both diffusion modes is significantly different from zero. Pair energy transport inhibits the potential's growth in the coupled case. The large build-up for the decoupled case may indicate steep chemical potential gradients opposite to those of temperature if a transition from coupling to decoupling occurs.

The modifications on the hydrodynamics of supernovae that may be implied by these results are difficult to predict. An order of magnitude estimate of the energetic differences that could arise was obtained by continuing the above computations to 7.5 ms. For purposes of comparison with extant supernova computations a similar diffusion study was carried out for coupled transport subject to a fixed zero chemical potential condition. The net energy and lepton number escape from the structure for the three different cases were as shown in Table 1.

Table 1 Net energy and lepton number escape

	Energy (erg)	Lepton number ($N_{\text{avg}} - g$)
Coupled	8.6×10^{51}	2.7×10^{32}
Decoupled	2.7×10^{51}	3.3×10^{32}
Zero chemical potential	3.2×10^{51}	—

The case in which a zero chemical potential is imposed most closely resembles the decoupled mode as far as energy is concerned. If a transition from coupled to decoupled diffusion occurs in actual supernovae, energy may accumulate at the transition point because of differences in diffusion rate. The above energy difference of $\sim 5 \times 10^{51} \text{ erg}$ is more than sufficient to give a type II supernovae. The possibility of such an accumulation of energy in the outer regions needs to be carefully considered.

These preliminary results indicate that the effects of lepton conservation may be significant in supernova neutrino transport. The validity of the diffusion approximation becomes questionable at lower temperature and density because of the long mean free paths of low energy neutrinos. The portion of the equilibrium neutrino spectrum thus affected is, however, relatively small over most of the collapsing supernova structure. Thus the general features of leptonic transport should be reflected in the results presented. The large magnitude of leptonic effects in diffusion shows that they cannot be ignored *a priori*. An extension of the multi-energy group transport approximation⁹ to allow for these effects is desirable.

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Measurement of atomic oxygen in the lower ionosphere using a rocket-borne resonance lamp

ATOMIC oxygen is an important constituent of the mesosphere and lower thermosphere because of its role in the excitation of the $O\ I(^1S_0-^1D_2)$ airglow emission at 557.7 nm (ref. 1) and because of its involvement in the positive and negative ion chemistry of the lower ionosphere². In particular it destroys negative ions³ and can break reaction chains believed to produce water cluster ions below about 85 km (ref. 4). There is also uncertainty about the degree of dissociation of oxygen in the thermosphere^{5,6} and atomic oxygen is involved in many reactions with neutral hydrogen/oxygen compounds in the mesosphere⁷.

Various experimental techniques have been used to measure the concentration of atomic oxygen [O] (refs 8-12) but most of these have serious limitations or uncertainties. We report direct measurements of night-time concentrations of atomic oxygen in the ground state, $O(^3P)$, in the mesosphere and lower thermosphere by a new technique. Values of [O] from 3×10^7 to $5 \times 10^{11}\text{ cm}^{-3}$ have been found at heights between 79 and 142 km. The experiments were rocket-borne, and used resonance fluorescence and absorption techniques at the $O\ I(^3P-^3S_1)$ triplet, 130.20, 130.47 and 130.58 nm.

O I radiation was produced in the rocket payload by a resonance lamp of novel design. It consisted of a sealed coaxial radiofrequency discharge tube within which molecular oxygen was produced by a temperature controlled chemical source, balanced by a getter. The oxygen pressure was adjusted to give minimal self absorption of the 130 nm radiation by atomic oxygen in the discharge. This was determined by laboratory measurements of the relative intensities of the components of the triplet, which dominated the spectral output of the lamp at $10^{13}\text{ photons s}^{-1}\text{ sr}^{-1}$. The lamp also radiated at H Lyman- α (121.6 nm), at the $O\ I(^3S-^3P)$ 136 nm lines, and at longer wavelengths.

The emission from the lamp was concentrated by a concave cylindrical mirror into a flat beam in the plane perpendicular to the rocket axis. A small part of the beam was reflected back by a corner reflector deployed on a boom and was detected by an ion chamber to measure absorption by ambient atomic oxygen over a 40 cm path. The ion chamber has a CaF_2 window and NO gas filling and was operated at a gas gain of about 600. Radiation at 130 nm was the only emission from the lamp within the bandpass of the detector (123 to 134 nm).

A photomultiplier was used to count photons re-emitted by the ambient oxygen atoms due to resonance fluorescence under

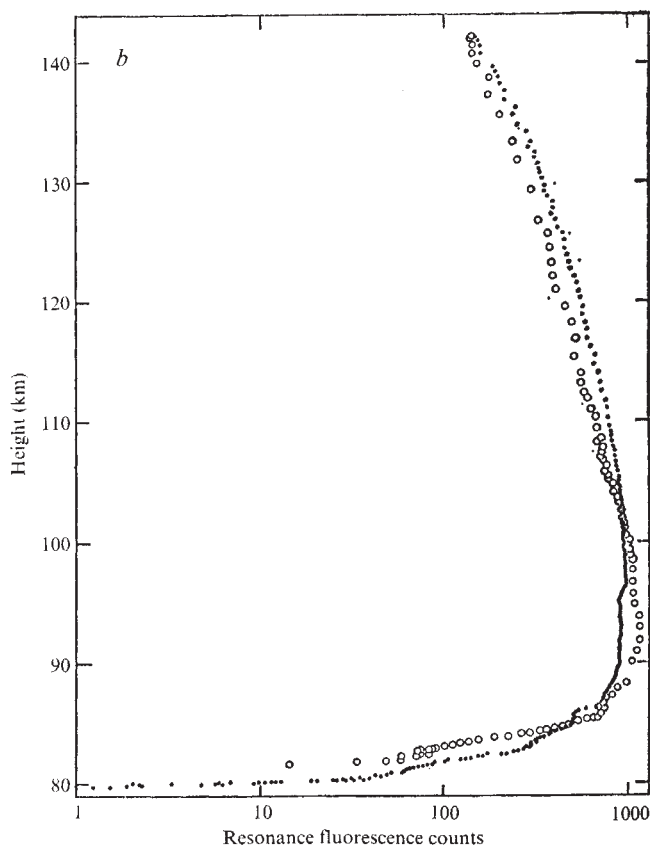
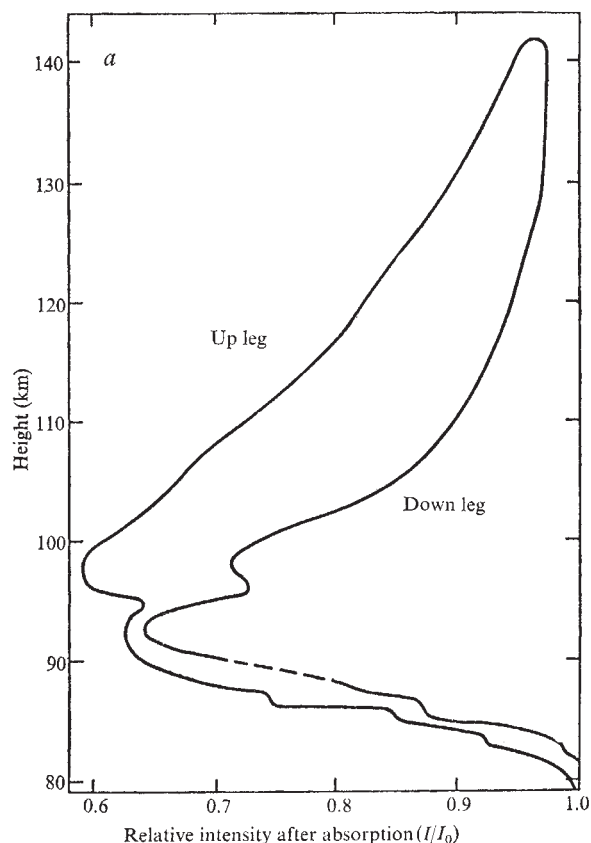


Fig. 1 *a*, Absorption of O I (130 nm) radiation over a path length of 40 cm through ambient atomic oxygen as a function of height for the complete rocket flight. *b*, Simultaneous measurements of the intensity of resonance fluorescence emission at $\lambda = 130\text{ nm}$ from ambient atomic oxygen as a function of height. ●, Up leg; ○, down leg.

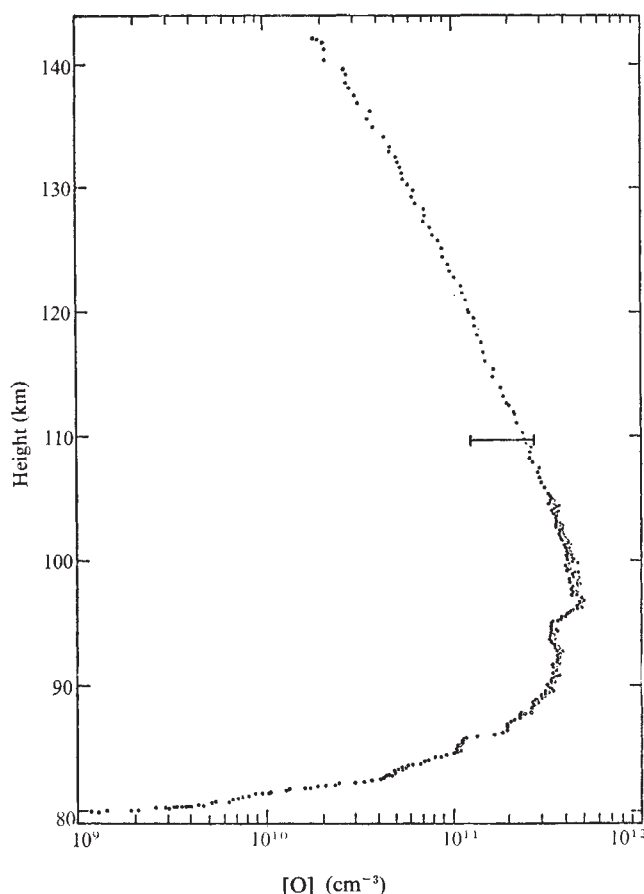


Fig. 2 Atomic oxygen concentrations calculated from the absorption and resonance fluorescence results as a function of height (up-leg data only). The error bar indicates the maximum range of uncertainty in the absolute scale.

irradiation by the ultraviolet beam. The lamp intensity was square wave modulated at 240 Hz so that phase-sensitive detection could be used to reject background signals due to airglow. A CaF_2 window was again used to suppress Lyman- α background. The field of view of the photomultiplier excluded all payload components.

The concentration of atomic oxygen which was required to produce a given degree of absorption was determined in the laboratory by measuring across a flowing afterglow in which known atomic oxygen concentrations were produced. This allowed absolute values of [O] in the ionosphere to be found from the absorption experiment in flight.

The resonance fluorescence experiment was not used to obtain independent absolute measurements of [O] because knowledge of all factors affecting the optical efficiency of the experiment would have been required, resulting in a relatively large uncertainty. But absolute values obtained from the absorption experiment are independent of these factors and were used to calibrate the resonance fluorescence for [O] values in the approximate range 2×10^{10} to $5 \times 10^{11} \text{ cm}^{-3}$. This calibration indicated some non-linearity in the resonance fluorescence count response at high count rates which was consistent with the known characteristics of the electronics as measured in the laboratory. At lower count rates a linear dependence of count rate on [O] was obtained and used to extrapolate the calibrations to [O] values below the range of sensitivity of the absorption experiment.

Two payloads, each including the above experiments and a Langmuir probe, and longitudinal and transverse magnetometers, have been flown on Petrel rockets. Rocket number

P174H was launched at night and P173H at dawn. Each flight was followed within 20 min by a second Petrel rocket carrying a support payload to measure atomic oxygen using resistive silver film sensors⁶ and electron density by Faraday rotation and Langmuir probe. Useful results were obtained from all four flights.

This letter deals solely with atomic oxygen concentrations from P174H. The flight was made from South Uist, Scotland ($57^\circ 20' \text{N}$, $7^\circ 20' \text{W}$) at 2237 UT on April 1, 1974, under magnetically quiet conditions. It reached an apogee of 142 km and was spin stabilised. The experiments were all operating from 64 km upwards. The trajectory was determined from Doppler slant range measurements and radar tracking, and is accurate to better than $\pm 0.5 \text{ km}$ in height.

The dependence of the measured absorption on height is shown in Fig. 1a. This may be compared with the counts from the resonance fluorescence in Fig. 1b, each point being the average of six counts sampled at 240 Hz. The derived atomic oxygen concentrations are shown in Fig. 2 for the up-leg data only.

In calculating the effective length of the absorption path in flight, molecular flow and complete recombination of oxygen atoms at surfaces have been assumed. If the surface recombination were low a stagnation zone of high [O] in part of the absorption path might cause an overestimation of the ambient concentration. In Fig. 2 this unlikely case is represented by the minimum density end of the error bar which indicates the range of uncertainty in the absolute [O] scale.

The atomic oxygen concentration was below $3 \times 10^7 \text{ cm}^{-3}$, the lowest detectable value, until 79.6 km was reached. There an extremely rapid monotonic rise by a factor of 40 in 0.4 km occurred, which has been omitted from Fig. 2 to avoid undue contraction of the [O] axis. (The omitted data can be seen in Fig. 1b below about 10 counts.)

Between 80 and 97 km, [O] increased and the measurements showed departures from a smooth variation. These occurred between 82.5 and 83.5 km, between 84.8 and 87 km and between 93 and 96 km. These perturbations were detected by both the absorption and resonance fluorescence experiments on the up and down legs of the flight at the same heights. They are, therefore, considered to indicate fine structure in the variation of [O] with height and not an instrumental effect.

Above the maximum [O] at 97 km the fall off became approximately exponential with a scale height of $14.9 \pm 0.6 \text{ km}$.

On the down leg the resonance fluorescence response was lower and the absorption considerably lower than on the up leg, except at the down-leg maximum near 93 km where both responses were similar to the up leg. A reduced response was expected on the down-leg because of wake effects.

The rocket velocity was high enough to cause serious loss of resonance due to the Doppler effect if there were a significant component of the velocity along the ultraviolet beam. The beam geometry had therefore been chosen to make this error negligible on the up leg. But on the down leg, roll modulation was observed which may be attributed to the Doppler effect as predicted.

The concentrations shown in Fig. 2 are derived from up leg data only, for which attitude corrections are expected to be small except near apogee.

The results have been produced by analysis of ultraviolet paper records of the analogue data. We intend to publish fuller details of the experiment and more precise results from computer analysis of digital records later.

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which also provided the support payloads. Assistance with analysis of flight data was provided by Md Iqbal Ahmed, Commonwealth Academic Staff Fellow, Andhra University, Waltair, India.

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Thermospheric winds from laser tracking of sodium clouds

NEUTRAL wind measurements in the thermosphere have been made on many occasions using ground based photographic¹ or photometric observations² of artificial clouds released from rockets. These clouds are visible either through a chemiluminous reaction, such as with trimethyl aluminium (TMA) releases, enabling night-time observations to be made at altitudes between 80 and 150 km or through resonance emissions of sodium, lithium, barium or aluminium oxide when clouds are illuminated by sunlight, enabling twilight or daytime observations to be made above about 80 km.

To measure night-time winds at all heights above 80 km, in particular above 150 km, the normal limit of a TMA trail, we

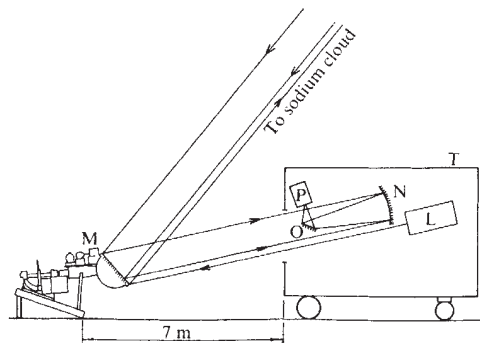


Fig. 1 Steerable laser radar. L, Rhodamine 6G flashlamp-pumped dye laser; M, steerable plane mirror (0.6 m × 0.4 m), N, O, Newtonian receiving telescope (aperture diameter 0.3 m), P, photomultiplier, processing and recording electronics; T, equipment trailer.

Table 1 Parameters of the steerable laser radar as used for rocket P146A

Transmitter	
No. of transmitted photons per pulse	1.4×10^{17}
Laser pulse repetition frequency	5 Hz
Wavelength	589.0 nm
Linewidth	3 pm
Beam divergence	2.5 mrad
Receiver	
Overall efficiency	0.04
Beam divergence	5 mrad
Raster scan	
Size	$20^\circ \times 20^\circ$ or $20^\circ \times 40^\circ$
Time for one raster	100 s or 200 s

have built a new mobile laser radar system which is used to track sodium clouds released from rockets. This letter reports the operation of the system during the United Kingdom high latitude rocket campaign at Andenes, Norway, in October and November 1973 and gives the wind profile obtained during one of the four successful flights.

The steerable laser radar (Fig. 1) was developed at the Appleton Laboratory from a fixed system previously used for studying the natural sodium layer by means of resonance scattering of a tuned laser beam³. The plane mirror, which is used to scan the laser beam around the sky, rotates about two axes, allowing the system to cover nearly half the sky. The scan is controlled

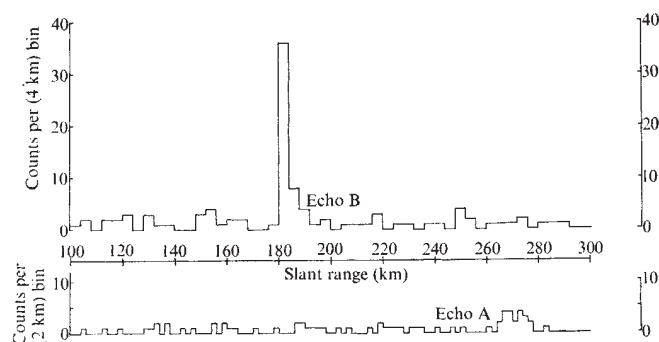


Fig. 2 Echoes from the sodium trail. Echo A (pulse No. 1007) is at 174.5 km altitude, 288 s after launch. Echo B (pulse No. 2885) is at 141 km altitude, 667 s after launch. The probability that the dark current and background signals, which should obey Poisson statistics, could produce a noise signal equivalent to these echoes is very small.

remotely in either manual or automatic modes. In the latter, a selected portion of the sky can be scanned in a raster. After each laser pulse, the photons detected by the photomultiplier are counted as a function of time delay and therefore slant range. The counts from each of 100 successive slant range intervals (where the intervals can be chosen to correspond to 0.5, 1, 2, 4 or 8 km), a time code and the angular coordinates of the scanning mirror are recorded in a suitable format on a digital cassette recorder for subsequent computer analysis. In addition analogue signals are reconstituted from the digital data to provide a quick-look record on an ultraviolet oscilloscope for immediate analysis and also a real time display on a cathode ray tube of intensity of echo as a function of slant range for each laser pulse. The location of the detected cloud within a given raster is stored and displayed continuously on a second cathode ray tube. This real time information on position, intensity and slant range of the cloud echoes enables the operator to optimise the raster location and the slant range settings of the recording system for subsequent rasters.

The system was deployed at Skibotn (69° 22'N, 20° 18'E) about 170 km from the rocket range at Andenes (69° 18'N,

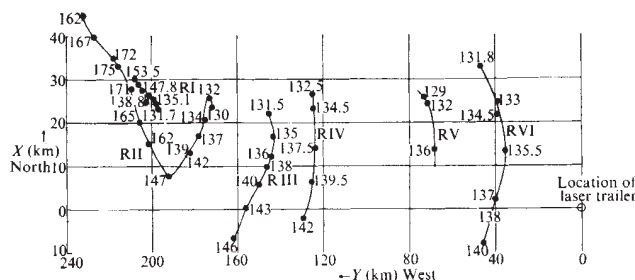


Fig. 3 Ground projection of sodium trial I to VI, Raster number; altitudes (km) are marked. The coordinates and altitudes shown are means, derived from analysing between 20 echoes (Raster I) and 160 echoes (Raster IV) per raster.

16° 02'E). Sodium thermite burners which would release a sodium cloud in the form of a trail were launched on four Petrel rockets (P100A, P101A, P145A, P146A) and as part of the payload of one Skylark rocket (SL1123). Rockets P100A (October 14, 1973) and P101A (October 17, 1973) were used to provide initial tests of the system for developing operational techniques. SL1123, followed by P145A and P146A were launched on the night of November 16/17, 1973 at intervals of approximately 1 h during a strong auroral substorm. Four of the five sodium trail releases were acquired and tracked, the one failure (P101A) being because of a partial rocket malfunction which caused a very low apogee.

The data presented here were obtained from P146A which was launched at 0101:02 UT on November 17, 1973, into the recovery phase of an auroral substorm. The sodium trail started near 130 km on the up leg, continued over apogee (175 km) and rather weak outgassing of sodium vapour continued to about 160 km on descent (the apogee and descent portions of the trail appeared from the laser echoes to be optically thin). The laser radar was operated for 35 min after the trail was released with the system parameters given in Table 1. 1,500 echoes from the trail were recorded from 10,000 laser pulses. Figure 2 shows two echoes. From the intensity of echo B at an altitude of 141 km it was deduced that the trail was probably optically thick. The return from 174.5 km (echo A) is from a well diffused and optically thin region near apogee.

Figure 3 shows the projection of the sodium cloud on to the Earth's surface (geocentric coordinates) as observed with the first six rasters. An average wind profile, Fig. 4, was deduced by following the projected ground track of various altitudes between the rasters.

Strong eastward and southward wind components above 130 km seen during the experiment are probably consistent with those to be expected from a combination of ion drag accelera-

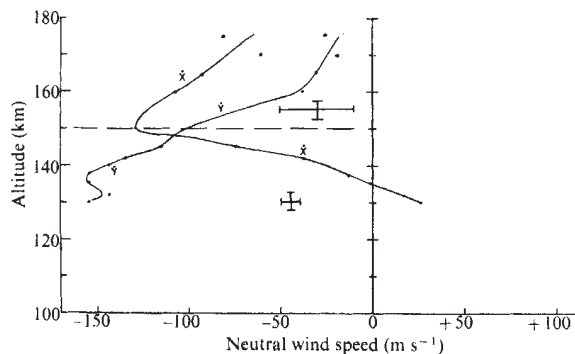


Fig. 4 Average wind profile for P146A. $\bar{X}$, $\bar{Y}$, Northward and westward components of velocity. Error bars indicate the maximum errors (± 3 standard deviations) in the two regions above and below -150 km.

tion and extensive heating in the auroral thermosphere during the auroral substorm of magnitude $\sim 500\gamma$ (nT) at Andenes, earlier in the night. The significance of these results regarding thermospheric morphology during auroral substorms will be studied using the results of all three rockets (SL1123, P145A, (146A) launched during the substorm.

During this campaign the greatest altitude at which echoes and wind data were obtained was the apogee of the Skylark (215 km). From the intensity and duration of the signal received at 215 km, we estimate that the laser radar system we used for these experiments can obtain wind profiles from sodium clouds at over 300 km altitude, the predicted design limit. This technique gives a considerable extension of the upper altitude limits at which night-time wind measurements can be made from rocket releases.

We thank the staff of the Auroral Observatory, Trömsö, for assistance in installing and maintaining the laser radar trailer at Skibotn. This was a collaborative project between University College, London, and the Appleton Laboratory of the Science Research Council. The rocket launches and the development work were supported by the Science Research Council. Mr E. Hammond helped to construct and operate the laser radar.

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Atmospheric halocarbons and stratospheric ozone

ODD chlorine¹ is a more potent catalyst for the destruction of ozone than is odd nitrogen². Molina and Rowland³ warn that chlorine released during the photochemical destruction of fluorocarbons (FCCs) constitutes a potentially serious threat to the integrity of the stratospheric ozone layer.

In the original investigations of the atmospheric distribution of FCCs (refs 4-7) these compounds were sought because of their unique usefulness as easily detected and unequivocal anthropogenic markers. Their presence in an air mass at elevated levels indicated its recent passage over an urban industrial region. The discovery of other halocarbons, accidentally revealed by the analysis, although scientifically interesting, was not the prime motive. So the literature on this topic gives the unintended impression that the FCCs are the predominant chlorine carriers in the atmosphere. In fact they are less abundant than other halocarbon classes and their presence might not have been discovered but for the exquisite sensitivity of the analytical method used.

Table 1 lists the observed abundances of the halocarbons at Adrigole in western Ireland in June and July 1974 in air coming from the west and in mid Atlantic aboard the research vessel Meteor in its cruise from Hamburg to Santo Domingo

Table 1 Background concentration of atmospheric halocarbons

Site of measurements	FCCs		Chlorocarbons			
	CCl ₂ F ₂	CCl ₃ F	CHCl ₃	CH ₃ CCl ₃	CCl ₄	CHCl ₃ = CCl ₂
Western Ireland	101.7	79.8	26.5	64.8	110.9	15.0
June/July 1974	(27.3)	(4.9)	(7.7)	(17.2)	(10.7)	(12.1)
North Atlantic	115.2	88.6	18.9	75.1	137.6	<5
October 1973	(33.1)	(4.05)	(14.7)	(9.2)	(14.7)	(5.7)
Chlorine equivalent	470				895	

Concentrations including Cl equivalent all in parts per 10¹² by volume, figures within brackets are standard deviations. Note: CCl₂ = CCl₂ has been characterised by retention time coincidence only.

in October 1973. Figure 1 illustrates the profile of concentration of CCl₃F and CCl₄ in the troposphere and lower stratosphere over the United Kingdom in June 1974.

The chlorocarbons are less chemically stable than the FCCs but this seems not to hinder their transport to the stratosphere. If we assume that the abundancies listed in Table 1 are representative of the troposphere generally then the proportion of the total chlorine transferred to the stratosphere by each compound will be in proportion to their mixing ratios and chlorine contents. These proportions are listed for each compound in the third row of Table 1 and suggest a Cl concentration at the tropopause of at least 1.4×10^{-9} . This is more than ten times larger than the estimate of inorganic Cl for this region^{8,9}.

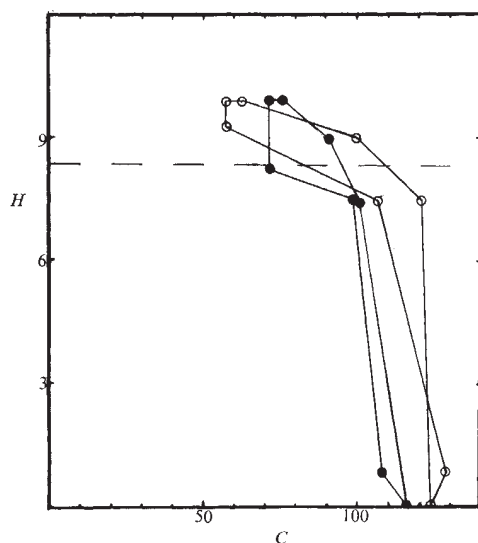


Fig. 1 Concentration (C) in parts per 10¹² by volume of CCl₃F (●) and of CCl₄ (○) over central England on June 6, 1974 at heights (H) in km. — —, Height of tropopause.

The FCCs seemingly contribute 35% of the total halocarbon chlorine entering the stratosphere. It is probable that this estimate is too high, since Table 1 lists only those compounds which have been observed. It is a commonplace for an unjustifiable significance to be attached to the easily measured quantity. It happens that the analytical method used is highly sensitive for CCl₃F and CCl₄. By contrast CH₂Cl₂ and CH₃Cl have not yet been sought because they are more difficult to detect. It would be sensible to determine the total organic chlorine content of the troposphere. It seems also probable that other chlorine compounds are there.

At first glance there would seem to be nothing unusual about the presence of traces of CCl₄ in the air. It is a typical organochlorine pollutant, a product of chemical industry. Closer investigation reveals that the origins of CCl₄ are tantalisingly

obscure. R. L. McCarthy (personal communication) estimates that the sum total of CCl₄ released to the atmosphere by all manufacturers and users of this compound does not exceed 10⁵ ton up to the end of 1972. This quantity, had it accumulated without loss, would have given an atmospheric concentration of 2.5×10^{-12} . This is forty times less than the present abundance—moreover, CCl₄ does not accumulate without loss; the soil, the sea and the stratosphere are all sinks^{5,10}. There may be some unlisted or incidental production and release of CCl₄ in industrial processes where chlorine encounters carbonaceous material. It seems unlikely that these unintentional sources are large; if they were it would surely be recognised, if only as a serious health hazard.

What then is the source of CCl₄? Marine algae emit halocarbons⁵ and a biological source of chlorocarbons is possible but its strength is unknown. A natural, probably atmospheric, origin of CCl₄ must also be considered as follows:

- 1) The abundance of CCl₄ does not differ appreciably between the Northern and Southern Hemispheres^{8,6}. This is not consistent with a northern industrial source.
- 2) Air arriving at western Ireland shows a strong correlation between the concentration of the FCCs and Continental European origin. No such correlation is observed with CCl₄.
- 3) Preliminary laboratory experiments in glass vessels show that the reaction in air between CH₄ and Cl₂ each at a concentration of 10⁻⁴ results in the production of small but significant quantities of CCl₄.

This evidence suggests a natural origin in the atmosphere itself. Chemical kinetics does not favour the direct gas phase chlorination of methane in the atmosphere but a heterogeneous atmospheric source is possible. If this is confirmed then other, as yet undiscovered, intermediates such as CH₃Cl and CH₂Cl₂ are likely also to be present. There is some indication from their spatial distribution that CHCl₃ and CH₃CCl₃ may also in part at least have a similar natural origin to CCl₄.

Molina and Rowland rightly warn of a potential hazard to stratospheric ozone should the emissions of the FCCs continue to grow unchecked but the prompt release of chlorine in the stratosphere from the other, non-FCC, halocarbons implies that the present input of odd chlorine species to the stratosphere is dominated by the simple chlorocarbons CCl₄, CHCl₃ and CH₃CCl₃ and so on. Indeed this input applied to the calculations of Wofsy and McElroy¹ indicates a 2% reduction of stratospheric ozone attributable to their presence. Unless there is some special additional effect from the release of chlorine at higher altitudes there are no grounds for singling out the FCCs as more hazardous than the other halocarbons which penetrate the tropopause.

In our paper on the distribution of halocarbons over the Atlantic⁵ we unwisely commented: "The presence of these compounds (the FCCs) constitutes no conceivable hazard". It may be as unwise to assume that we are now at the early stages of a serious global pollution incident. We need urgently to discover the sources and sinks of CCl₄ and similar chlorocarbons, and also the stratospheric sinks for odd chlorine. If there is a sizeable natural chlorocarbon cycle it will give, so to speak, a stay of execution.

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Accuracy of weather forecasts

AFTER nine years of publication there has been considerable criticism of the usefulness and accuracy of long range weather forecasts. Gordon¹ has suggested that the forecasts have not yet attained a practical or economical degree of usefulness, and that advance computer technology and statistical power will not improve their accuracy unless a breakthrough in ideas is achieved. Existing methods are broadly based on an analogue or matching technique in which overall weather circulations of a particular month for many decades in the past are compared with those of the month preceding that for which the forecast is to be made.

It is, therefore, clearly necessary that various assessments of the results are made independently of those calculated by the predictors.

I have devised a classification to evaluate the accuracy of the predictions for the London area, from observations made at Kew Observatory. The portion of the long range forecast used is that part which refers to southern or south-eastern England. Although the forecasts mention several parameters, I have selected temperature and rainfall for verification (probably the two most important factors for the consumer, whether in the public or private sector of the national economy). This study deals with the 108 months from June 1965 to May 1974, inclusive.

Table 1 Assessment of official long range forecasts

Predicted	Observed	Good	Bad	Very bad	Actual distribution
Above average	Temperature	15	10	10	42
	Rainfall	3	5	9	31
Average	Temperature	6	32		22
	Rainfall	22	36		38
Below average	Temperature	16	6	13	44
	Rainfall	13	11	9	39

Table 2 Scoring in systems A and B

All forecast above or below average		(assuming the expected distribution of 2, 1, 2 in 5 trials)	All forecast average	
A				
$2 \times 3 = 6$			$2 \times -2 = -4$	
$1 \times -2 = -2$			$1 \times 8 = 8$	
$2 \times -2 = -4$			$2 \times -2 = -4$	
Total	0			0
B	$2 \times 4 = 8$		$2 \times -2 = -4$	
	$1 \times -2 = -2$		$1 \times 8 = 8$	
	$2 \times -3 = -6$		$2 \times -2 = -4$	
	Total	0		0

The forecasts predict mainly one of three probabilities, below average, average (which includes near average or about average), and above average. A few forecasts of temperature have predicted much above average or much below average but these classifications have been treated simply as above average or below average in this study.

The boundaries which separate the classes above average, average, and below average are not published with the forecasts in the press or in the Weather Log (in *Weather*) and in many cases the public must make an inspired personal judgment. The Meteorological Office will, however, supply this information on request and it is understood that subscribers to the long range forecasts are provided with the exact boundaries. These are quintiles for temperature and terciles for rainfall, and are based on the climatic mean for the particular month and area of the United Kingdom for which the forecast applies. In the case of rainfall, $\pm 20\%$ of the mean climatic total defines the

Table 3 Sequential annual point totals 1965-74, inclusive (108 cases)

Year	Temperature		Rainfall	
	A	B	A	B
1965*	1	3	-1	-1
1966	16	19	3	2
1967	-4	-2	-3	-4
1968	-9	-10	-3	-4
1969	6	8	3	5
1970	21	27	3	1
1971	-14	-17	0	0
1972	-9	-11	3	5
1973	-9	-10	3	5
1974†	0	0	-2	-5
Total	-1	7	6	4

* 7 months.

† 5 months.

middle tercile or band width for the average condition. In the case of temperature the middle quintile for Kew is about $\pm 0.35^\circ\text{C}$ when averaged throughout the year; it is used in the forecast assessment to define the average condition.

The classification system used here to assess the forecast results considers nine boxes, three for each class of prediction. If the observed result falls in the same box as the predicted result the forecast is 'good', if in a box adjacent to the predicted result the forecast is 'bad', and if in a box two boxes away from the predicted result the forecast is 'very bad'. In the last case it means that a forecast for above or below average conditions has been made whereas the observed result has been the opposite, that is, below or above average, respectively.

Table 1 shows the results for temperature and rainfall, together with the actual distribution of observed results. The

observed quintile distribution follows closely the ratios 2,1,2 where much above and much below average are included with the above and below average cases. The observed tercile distribution follows the 1,1,1 ratios acceptably well for the rainfall.

Two scoring systems may be used. The first is that applicable to a game of chance. The second is rather better for the purpose of testing a scientific prediction technique in that it gives a one point bonus for a correct above average or below average forecast and a one point penalty for a very bad forecast as already defined.

For temperature, the quintile distribution is divided into the proportions 2,1,2. The probabilities for each class above average, average and below average, respectively, are therefore 2/5, 1/5, and 2/5. These three probabilities are equivalent to odds of 1.5 to 1, 4 to 1, and 1.5 to 1, respectively (3 to 2, 8 to 2, and 3 to 2, respectively). The gains for a success are therefore 3 for above average, 8 for an average and 3 for a below average. A failure in any class loses 2 points and this is indicated by -2. That explains the scoring system if the rules of a game of chance are operated (system A).

Scoring system B is exactly the same as system A except for a modification which allows arbitrarily one point extra if a success is forecast for above average or below average. Thus, a success for above average is 4, for average 8 as before, and for below average 4. To equalise this a penalty of one point is invoked if the result is the opposite of the forecast of condition. For the latter case the loss is -3 instead of -2.

Both systems add up to zero if all cases are forecast in any one category (Table 2). The total scores for the nine-year period studied are:

Temperature: system A, -1; system B, 7. Rainfall: system A, 6; system B, 4.

None of the scores approaches an acceptable level of statistical significance as given by the chi square test for 108 cases. Wright and Flood² found no statistical significance for the temperature and rainfall forecasts in an assessment covering the results of forecasts for the whole country for a two-year period. Some statistical significance was, however, found for the general information forecasts. The latter comprise statements of the kind "soon becoming wetter", "dry weather at first", "some warm days", and so on. In view of the fact that such statements are often very subjective in character it is extremely difficult to assess the results quantitatively. For this reason, and because the predictions are often of a synoptic scale type and, therefore, not really long range forecasts, it is doubtful if the significance is meaningful in the context of a 30-d period.

The results of the long range forecasts for a month ahead for temperature and rainfall do not approach an acceptable level of statistical significance. In fact, it is doubtful whether one can honestly say that they are even marginally better than chance. Although the selected cases only represent a sample of the total population of cases which could include a further 10 areas, the London area could be considered as one of the most important for verification. Further studies on the other areas should be done but it is doubtful if the net result would show any marked improvement. Furthermore, it is extremely doubtful that Ratcliffe's claim³ of a modest but real improvement in the last few years in the long range forecasts is now valid. Table 3 shows the year-by-year scores for systems A and B for both temperature and rainfall.

It is also questionable whether the band width of $\pm 0.35^\circ\text{C}$ for the middle quintile for temperature is at all meaningful as a definition of average temperature for the consumer. A band width of $\pm 1^\circ\text{C}$ might be far more useful to the suppliers of gas, electricity, coal and so on, for planning purposes.

It is certainly time to consider whether the very considerable expense incurred by this branch of our National Meteorological Service should continue to support what might be judged as a profitless activity. Bearing in mind that it is recognised that there is a built-in persistence factor in the sequence of

meteorological conditions, almost any person conscious of the weather should be able to obtain a small positive score after a sufficient number of trials, given the previous months anomalies.

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Microstress mechanism for the time dependence of the modulus of crystalline polymers following an imposed change in volume

PARRY and Tabor¹ have observed a significant time dependence of the dynamic shear modulus (G') of crystalline polymers at high hydrostatic pressure (10,000 pound inch⁻²). The modulus observed a few seconds after the increase in pressure is lower than the modulus observed at later times. There are two obvious explanations of this effect. The first is that it is due to a time-dependent bulk compliance (B). A pressure-induced reduction in volume increases G' . If B is time dependent, then the volume will also be time dependent and will continue to decrease after the increase in pressure, thus rendering G' time dependent. The second explanation is that the time dependence of G' is due to the pressure-induced generation of shear stresses in the heterogeneous solid. Of these explanations Parry and Tabor¹ favour the second. This view is supported by the following argument.

It is known that in polycrystalline solids with anisotropic thermal expansion coefficients, a change in temperature produces profound changes in both the plastic properties of the solid and the creep compliance²⁻⁶. This effect is attributed to the thermally induced generation of microstresses. The volume change of each anisotropic unit is constrained by the aggregate so that shear stresses are induced. The shear stresses relax slowly with time as the units shear. If the solid is stressed at the time of the temperature change the effective stress is the sum of the applied stress tensor and the microstress tensor and it is this combination of stresses, together with the nonlinear properties of the solid, which produces the anomalous mechanical properties.

The preceding mechanism is independent of the sign of the imposed change in volume. Evidence that this is in accord with observation is shown in Fig. 1. A specimen of linear polyethylene (Hifax 1900) was heated to 50°C and maintained at that temperature for 12 h. It was then cooled slowly to 40°C . After 10 min the temperature was increased abruptly (within 1 s) by $\Delta T = +2.8^\circ\text{C}$. A series of stress pulses (of duration 100 s) was then applied at various times after the jump in temperature. The interval between the stress pulses was sufficiently long that the strain had recovered to zero before the next stress pulse was applied. A second experiment was then performed, identical to the first in every way except that the temperature was reduced abruptly by $\Delta T = -2.8^\circ\text{C}$. Fig. 1 shows the time dependence of the observed shear strains at 40 s, 1,000 s and 6 h after the abrupt change in temperature. For both positive and negative values of ΔT the creep strains decrease with storage time. That is to say, although the thermally induced

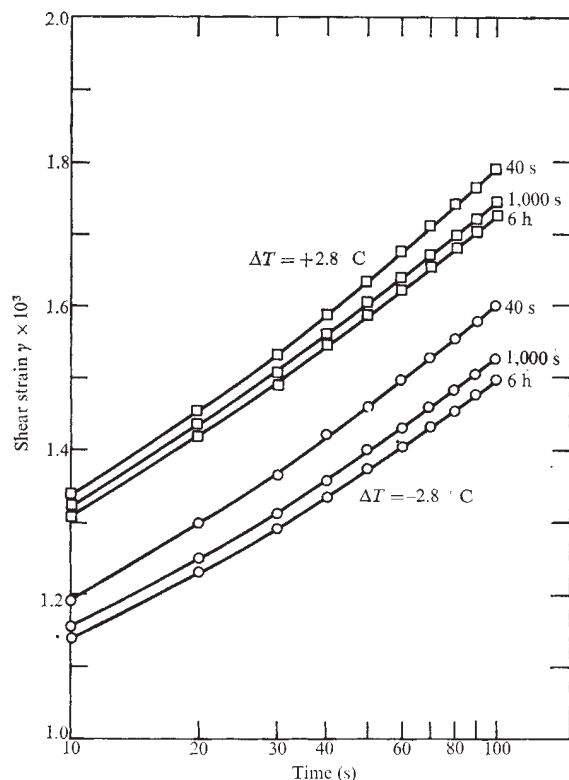


Fig. 1 Dependence of shear strain on time in successive 100-s pulse experiments for linear polyethylene showing the systematic reduction in strain with storage time for both positive and negative values of ΔT . Temperature cycle: $\uparrow 50^\circ\text{C}$; 12 h; $\downarrow 40^\circ\text{C}$; 10 min; ΔT ; pulse experiments. Shear stress $\sigma_0 = 0.175\text{ N mm}^{-2}$.

volume changes were positive and negative the effect of storage time is always to increase the modulus.

The preceding experiment disposes of alternative mechanisms, such as time dependence of volume. It also leads us to predict that if the interpretation of Parry and Tabor¹ is correct then the observed value of G' in their experiments will always increase with storage time after a pressure change, independent of the sign of Δp . The crucial experiment will be to determine the time dependence of G' following a negative Δp .

It will be noted that the effect of lapse time is more marked in Fig. 1 for $-\Delta T$ than for $+\Delta T$. This effect is due to the thermal history: the specimen was cooled to 40°C from 50°C . If the thermal cycle is altered in this respect alone ($\uparrow 50^\circ\text{C}$; $\downarrow 30^\circ\text{C}$; 12 h; $\uparrow 40^\circ\text{C}$; 10 min; ΔT ; pulse experiment) then the effect of lapse time is more marked for $+\Delta T$ than for $-\Delta T$. The explanation is that the specimen is to some extent equilibrated for either $+\Delta T$ or $-\Delta T$ depending on the direction of approach to 40°C before the jump in temperature.

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Effects of non-electrolytes on the solubilities of salts in water

It was shown by Setchenow^{1,2} that the solubility (s_w) of a non-electrolyte in an aqueous solution of salt concentration c_s was given by

$$\log s_w = \log s_0 + k_s c_s \quad (1)$$

where s_0 is the solubility of the non-electrolyte in pure water and k_s is a constant. Deno and Spink³ found that for a given salt, k_s was proportional to the characteristic volume of the non-electrolyte which in $\text{m}^3\text{ mol}^{-1}$ equals the parachor (calculated in the usual way⁴ in c.g.s. units) $\times 10^{-6}$. The effects on the solubilities of salts in water of the addition of many non-electrolytes is now shown to depend also on the characteristic volumes as well as on the concentrations of these non-electrolytes.

Rothmund⁵ found that equation (2) could be used for the solubilities of lithium carbonate in water containing non-electrolytes where S_w and S_0 are the solubilities of the salt in the solution of non-electrolyte (concentration C_s) and in pure water, respectively. Rothmund also measured the solubilities of silver sulphate, potassium bromate, potassium perchlorate and hydrated strontium hydroxide in water and in solutions (500 mol m^{-3}) of non-electrolytes and found that, in general, the order for the effect of non-electrolytes was the same for each salt. It has now been found that K_s is proportional to the characteristic volumes V^* of the non-electrolytes and that equation (3) can be applied to the solubilities of salts in aqueous solutions of many non-electrolytes.

$$\log S_w = \log S_0 + K_s C_s \quad (2)$$

$$\log S_0 \text{ (a constant for a given salt)} = \log S_w + k'_s V^* C_s \quad (3)$$

Weber⁶ carried out experiments similar to those of Rothmund. In Table 1 the results of Weber for potassium sulphate at 298.16 K have been used and, for the non-electrolytes listed, results for lithium carbonate^{5,6}, potassium bromate⁵, silver sulphate⁵, silver bromate⁷ and lead sulphate⁸⁻¹⁰ at 298.16 K are given. The values of k'_s used were derived from all results reported and not just from those listed in Table 1. As required by equation (3) values of $\log_{10} S_w + k'_s V^* C_s$ are indeed, in most cases equal to $\log_{10} S_0$ for a given salt. Some of the solutions of non-electrolyte are quite concentrated. For equation (3) to apply, the salt should be practically insoluble in the pure non-electrolyte which, of course, should not react chemically with the salt. Rothmund⁵ showed that silver ions complex with ammonia and with amines. From Table 1, it would seem that silver ions also combine with acetonitrile and with phenol.

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Table 1 Effects of non-electrolytes on solubilities of salts in water at 298.16 K

Non-electrolyte and its characteristic volume (V^* , $\text{m}^3 \text{mol}^{-1}$)	Formula of salt (value of k'_s)											
	KBrO ₃ (0.8)		AgBrO ₃ (0.6)		K ₂ SO ₄ (1.0)		Ag ₂ SO ₄ (1.1)		Li ₂ CO ₃ (1.05)		PbSO ₄ (1.6)	
	C_s (concentration of salt, mol m^{-3}) and corresponding value of $\log_{10} S_w + k'_s V^* C_s$											
None		2.679		0.910		2.745		1.427		$\left\{ \begin{matrix} 2.227 \\ 2.228 \end{matrix} \right\}$		$\left\{ \begin{matrix} -0.824 \\ -0.829 \end{matrix} \right\}$
Methanol (8.74×10^{-6})	500	2.682	3,064	0.902	125	2.745	500	1.444				
			6,021	0.895	250	2.746			250	2.228		
			8,890	0.889	500	2.743			500	2.230		
			11,620	0.867	1,000	2.742			1,000	2.236		
			14,240	0.840								
			16,680	0.794								
Ethanol (1.2735×10^{-4})	500	2.675	2,128	0.905	125	2.745	500	1.428	125	2.225	811	-0.830
			4,198	0.908	250	2.747			250	2.225	1,732	-0.864
			6,188	0.917	500	2.743			500	2.218	2,128	-0.834
			8,090	0.913	1,000	2.732			1,000	2.214	4,198	-0.823
			9,880	0.889							6,188	-0.858
											8,090	-0.953
											9,880	-0.782
<i>n</i> -Propanol (1.673×10^{-4})	500	2.679	1,636	0.927	125	2.746	500	1.431	125	2.227		
			3,220	0.963	250	2.747			250	2.227		
			4,730	0.995	500	2.744			500	2.228		
			6,170	1.001	1,000	2.754			1,000	2.216		
			7,540	0.966								
<i>t</i> -Amyl alcohol (2.472×10^{-4})	500	2.682			125	2.745	500	1.444	125	2.227		
					250	2.743			250	2.224		
					500	2.741			500	2.218		
					1,000	2.746			1,000	2.213		
					125	2.743			62.5	2.226		
<i>i</i> -Amyl alcohol (2.472×10^{-4})									125	2.225		
Acetone (1.551×10^{-4})	500	2.690	1,696	0.930	125	2.745	500	1.428	125	2.225	699	-0.862
			3,343	0.944	250	2.745			250	2.221	1,413	-0.948
			4,925	0.935	500	2.738			500	2.222		
			6,440	0.907	1,000	2.738			1,000	2.206		
Butan-2-one (1.9505×10^{-4})					125	2.749						
					250	2.750						
					500	2.755						
					1,000	2.769						
Diethylamine (2.189×10^{-4})	500	2.672			125	2.751			125	2.230		
					250	2.757			250	2.228		
					500	2.774			500	2.223		
					1,000	2.783			1,000	2.202		
Acetonitrile (1.146×10^{-4})					125	2.747	500	1.783	125	2.224		
					250	2.750			250	2.222		
					1,000	2.751			500	2.215		
									1,000	2.191		
Paraldehyde (2.8975×10^{-4})					125	2.755			62.5	2.231		
					250	2.765			125	2.235		
					500	2.777			250	2.242		
Phenol (2.1975×10^{-4})	500	2.717			125	2.751	500	1.700				
					250	2.757						
					500	2.769						
Methyl acetate (1.7175×10^{-4})	500	2.692			125	2.755	500	1.421				
					250	2.763						
					500	2.779						
					1,000	2.809						
Ethyl acetate (2.117×10^{-4})					125	2.756						
					250	2.768						
					500	2.790						

Maternal nutrition and the sex ratio at birth

MANY experiments on laboratory animals have shown that the nutrition of the dam can affect both litter size and viability. An early study indicated that breeding pairs of rats fed a poor diet also had an altered sex ratio in their offspring¹, but no more precise nutritional studies of this have been made. Nutritional factors have not been considered amongst the determinants of the sex ratio at birth.

Female albino mice (Tuck and Sons, Essex) aged 35 d were

divided into two weight-matched groups and fed semipurified diets: either a low fat (LF) diet or a control diet. The diets differed only in their lipid composition. The control diet contained 66 g kg⁻¹ lipid, the low-fat diet 6 g kg⁻¹. In the control diet, 5.0% of the dietary energy was derived from linoleic acid, 1.0% from linolenic acid. In the low-fat diet 0.27% of the dietary energy was as linoleic, 0.06% as linolenic acid².

The control diet has been shown in this laboratory to support rats and mice through three generations, the low-fat diet to

induce essential fatty acid (EFA) deficiency in rats and mice. Animals were housed at 27°C (range 26–28°C) at a relative humidity of 80% (range 70–90%).

After 12 weeks on the diet, the animals were mated with males of the same strain that had been fed a standard laboratory diet (Diet 86, Dixons Ltd). Two females were caged with each male for 7 d during which time the males received the same diet as the females. Each male was used for only one mating.

Pregnant dams were caged individually 20 d after the introduction of the male and cages examined at least once a day for the presence of litters. The experiment was repeated three times.

The results presented in Table 1 show that still births and cannibalism were increased and litter size reduced on the low-fat diet, a result consistent with previous reports^{2,3}.

What has not been previously demonstrated, however, is that the marked reduction in litter size is attributable almost completely to a reduction in the number of male pups born, no significant change occurring in the number of female births. The deviation in sex ratio at birth was due to this deficit in males relative to the control group and not to any excess in females. In spite of variations in litter size, in no litter born to a female on the LF diet was there an excess of male over female births. The unlikely possibility of parthenogenesis has been suggested when such sex ratios have been observed in mice, but this has found no experimental support⁴. Thus, either the Y sperm were less efficacious, or male foetuses were less viable *in utero*. The former possibility is unlikely, as EFA deficiency lesions take a considerable time to appear in animals fed a low-fat diet⁵ and the male mice only ate the LF diet for 7 d during mating.

which survived had a lower EFA requirement or were more favourably positioned *in utero*. We are investigating this possibility and meanwhile suggest that the selection of progeny by nutrient requirement could complicate the interpretation of multigenerational experiments involving EFA deprivation^{2,3}.

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Table 1 The effect of diet on the number of male and female offspring

	Maternal diet LF diet	Control diet	Difference	P
No. litters born	26	28		
No. litters stillborn*	6	1		$P < 0.05$
No. litters partly or totally cannibalised	12	2		$P < 0.01$
No. litters examined for sex ratio	8	15†		
No. pups per litter	5.13	7.94	2.81	< 0.025
No. male pups per litter	1.25	4.00	2.75	< 0.001
No. female pups per litter	3.88	3.94	0.06	n.s.
Sex ratio (males per 100 females)	32.2	101.5	69.3	< 0.05

Significance testing by χ^2 or Student's *t* test as appropriate.

*Dead at first examination.

†10 litters excluded because no usable litters born to LF females from that mating.

Adult male mice have a much higher EFA requirement than females⁵. The difference is presumed to be related to the known effects of sex hormones on EFA metabolism⁶. Some data suggest that the sex differences are not evident prior to sexual maturity⁷, although this point has been contested⁸. The results of our experiment suggest that a sex difference in the sensitivity to EFA deficiency is not only established before sexual maturity, but before birth.

Marked distortions in sex ratio in some strains of mice are known—in one study on an inbred strain a sex ratio of 62 males per 100 females was obtained, but the cause of this remains 'still obscure'⁴. We suggest that the possibility of an elevated EFA requirement in such strains should be investigated. Our results indicate that even in common laboratory mice, foetal deaths can apparently be related to nutrient requirement. Therefore, it is possible that those male foetuses

Two modes of evolution

IN some ways mammals evolve much faster than most other animals. In other ways however it now seems that they evolve at the standard rate. This dichotomy is quite unexpected, but seems *post facto* to be predicted by a new and ecological explanation of evolution.

Table 1 lists the phenomena whose relative rates are known. Faster turnover rates (origination and extinction) for mammals are well documented^{1,2} and can be shown in several ways not to be a taxonomic artifact (Van Valen, unpublished). Structural innovation and complexity is difficult to quantify, but it is commonly believed¹ to evolve unusually rapidly in mammals. The relative rates for chromosome number and hybrid inviability are from a comparison of frogs and mammals³.

The similar rate of amino acid substitution in proteins^{4,5} in mammals and other organisms is a well known problem. Its supplementation here with size evolution makes the problem worse. Kurtén⁶ found that for ordinarily studied intervals of mammalian evolution (his "Tertiary" distribution), rates of measurable characters have a central tendency of about 30 millidarwins (ref. 7). There are almost no estimates of such rates for fish, amphibians, invertebrates, protists or plants. Because growth and evolution occur largely on a proportional rather than an absolute scale, logarithmic rates are appropriate^{1,7}.

Table 1 Two modes of evolution in mammals with their associated phenomena

Mode	Phenomenon
Standard	Protein sequences Linear measurements (size)
Epistandard	Origination and extinction Structural innovation and complexity Chromosome number Hybrid inviability

For another purpose I calculated 13 rates for invertebrates from data of Newell⁸. Because he may have selected his examples for higher rates, I then took the first 15 suitable references from my card file of evolutionary series and calculated rates from their data. Time scales are mostly from the Geological Society of London⁹, Kauffman¹⁰, or the original authors. Table 2 shows the results; the rates from Newell's data are comparable to the rest.

Although there are some patterns in the rates of Table 2, as a rough estimate of the central tendency we can use the logarithmic (geometric) mean of all the 75 rates weighted

equally. This is about 40 millidarwins, ostensibly even a little higher than the value for mammals. (The variation in both distributions, however, is large enough for no biologically real difference to be apparent.) Two previous comparisons^{26,27} of size rates between invertebrates and mammals both gave invertebrates the higher rate, although each used less than a half dozen rates and neither noted the resulting paradox. The highest known rates of size evolution are similar for mammals⁶ and protists²⁸, about 20 darwins in each case (excluding human introductions).

Some mammalian structures participate in both modes of evolution and so may focus the problem. Horse teeth are an example. Their measurements evolve in the standard mode¹ but their patterns of enamel, dentine, and cement evolve in the epistandard mode, as judged directly but subjectively, and indirectly by the rates^{1,2} of origination and extinction of taxa that are defined in part by their tooth pattern.

The difference between the modes clearly is not that of neutral against adaptive evolution. Measurable aspects of horse teeth are of obvious selective importance; strong natural selection has in fact been measured on them²⁹. The degree of constancy for size evolution is nevertheless indistinguishable now from that of protein evolution, which has some more or less predictable deviations from constancy^{30,31} and which is measured on a much grosser scale than is size evolution. The Red queen's hypothesis^{2,31} predicts such a degree of constancy, and I have suggested³¹, without evidence, that fish have really evolved as fast as tetrapods.

How then to explain the epistandard mode? In the resource space³² the proportion of the resources used by one species changes over evolutionary time; usually, if one species gains resources, others lose. Presumably the boundaries in the resource space between species shift back and forth, rather than there being mostly unidirectional changes. The Red queen proposes that the changes occur at a constant average rate. The rate of extinction depends on the susceptibility of the species at risk: the greater the proportion of its resources it loses, the greater the risk of extinction.

For mammals, then, the spectral threshold³³ of ultimately regulatory resources needed to prevent extinction is presumably lower (loss of a lower average proportion of resources causes extinction) than in most organisms. Mammals then become extinct more often than most organisms with similar fluctuations in the partitioning of the resource space. Epistandard origination rates are needed to replace the extinctions; perhaps this just involves greater survival of incipient species, which could themselves help cause the extinctions. The apparently epistandard evolution of morphological pattern is clearly consistent with this situation, as is the epistandard reshuffling of the genome. The latter effects, however, may not be necessary causal adjuncts.

And why should mammals be so susceptible to extinction, or have such a high rate of formation or survival of incipient species? There are several possible explanations, related to mammalian attributes such as the high rate at which they use energy, their apparently sharply bounded but contiguous adaptive zones, the ease with which their genomes became reorganised, or their high-level feedback systems. Whatever the resolution of this point, it is clear that phenomena related to origination and extinction rates should participate in the epistandard mode.

Thus, for a group whose taxa have a low threshold for extinction, such as mammals, some phenomena will evolve in the epistandard mode, in some sort of causal relation to this low threshold. But other phenomena will be unaffected by the causal nexus of the threshold and will evolve in relation to the standard losses and gains in resources (realised fitness). Possibly some phenomena are intermediate, and other groups are expected to participate in epistandard mode to the extent that their extinction rates are above normal.

In the zero-sum game of the Red queen each species competes, directly or indirectly, with all others, and no species ever wins;

Table 2 Evolutionary rates for invertebrates and protists, based on measurements and meristic characters (counts)

Taxon	Character	Time Interval (millions of years)	Rate (milli-darwins)
Foraminifera			
<i>Bolivina</i> ¹¹	width	18	20,30
Ataxophragmiidae ¹¹	length	10	80
<i>Fronicularia</i> ¹¹	width	2	300
Gavelinellidae ¹²	diameter	20	20
<i>Lagena</i> ¹³	length	6	140
Orbitoides ¹⁴	early diameter	6,7	60,100
	number early chambers	6,7	90,120
<i>Globoconusa</i> ¹⁵	proloculus diameter	4	40
	final diameter	4	130
	number chambers	4	70
Fusulinidae ⁸	diameter	45	80
Nummulitidae ⁸	diameter	25	200
Bryozoa			
Fenestrellinidae ⁸	aperture width	25	20
Brachiopoda			
<i>Eocoelia</i> ¹⁶	width	5	90
	number ribs	5	90
<i>Dictyothyris</i> ¹⁷	length	5	90
<i>Cyrtospirifer</i> ¹⁸	number ribs	8,15	100,0
<i>Gypidula</i> ¹⁹	beak height	2	140
Terebratuloidae ⁸	diameter	40	40
Pentameroidae ⁸	diameter	20	50
Productacea ⁸	diameter	20	140
Richthofeniidae ⁸	depth	40	40
<i>Rafinesquina</i> ⁸	width	20	50
<i>Punctospirifer</i> ⁸	diameter	30	50
Pelecypoda			
<i>Gryphaea</i> ²⁰	width attachment	8	130
	width shell	8	160
<i>Argopecten</i> ²¹	height	10,8,8,7,2,8,8	140,60,30,10,90,120,50
	number costae	10,8,8,7,2,8,8	20,6,8,30,190,10,20
<i>Myalina</i> ⁸	hinge length	50	40
Ammonoidea			
Pinacoceratidae ⁸	diameter	35	40
Ostracoda			
<i>Cytherella</i> ²²	length	7	3
<i>Ovocytheridea</i> ²²	length	3	4
<i>Mehesella</i> ²²	length	15	30
<i>Leguminocythereis</i> ²²	length	3	70
<i>Cythereis</i> ²²	length	8	9
<i>Trachyleberis</i> ²²	length	3	9
<i>Buntonia</i> ²²	length	3,3,2,25	40,40,8,20
Stelleroidea ⁸	maximum width	100,350	15,5
Echinoidea			
<i>Micraster</i> ²³	length	18,18	8,20
Holasteridae ²⁴	length	5	260
	height	5	50
Melechinoidae ⁸	width	20	60
Graptoloidea			
Neocucullograptinae ²⁶	length sicula	5,5,1,2,1,3	20,30,190,40,50,80
	length first theca	5,5,1,2,1,3	4,30,160,140,140,30

Individual values are accurate only to about a factor of 2, but presumably are unbiased.

new adversaries replace the losers. This world is stark and shifting; wherever an equal shift causes greater harm, as for mammals, we have the possibility of explaining the additional mode of evolution that occurs there.

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Honey bee communication

MORE than fifty years ago von Frisch demonstrated that foraging honey bees aroused the interest of potential recruits by means of a 'dance'. These experiments showed that the forager's body provides sufficient information about the odour of the food source to enable other bees to find that food in the field. In addition von Frisch demonstrated that foragers also leave odours at the food source which aid recruits in their searches^{1,2}. Von Frisch later found that the dance also contains distance and direction information which the recruits seem able to use^{3,4}.

Recently von Frisch's controls have been criticised and new experiments have suggested that olfactory information alone is sufficient to explain the phenomenon of honey bee recruitment^{5–10}. These experiments, however, were also subject to criticism^{11,12} and have stimulated new work with improved controls for odour cues^{13–16}. These more recent results are still subject to the interpretation that the bees might have been

using a 'locality odour' instead¹⁷. Since there is evidence that bees can use such cues^{17,18}, interpretation of previous work is seriously complicated. The experiments reported here were designed to examine this question more rigorously.

Under some circumstances honey bees seem to interpret a bright light in the hive as the Sun and will orient their dances to it rather than to gravity. Since both dancers and dance attenders are reoriented, no misdirection occurs¹⁹. When the three ocelli are painted over, however, a honey bee becomes far less sensitive to light^{20,21} and when a light of an appropriate brightness is used, foragers with painted ocelli will ignore the light and dance with respect to gravity; while untreated bees both dance and interpret dances as though the light were the Sun. If the bees use the distance and direction information in the dance, it should be possible for ocelli-painted foragers to send recruit bees to specific but incorrect locations.

Two groups of foragers were trained using Gary's technique²². The first group served as a control to measure the degree of reorientation of the dances to the light. The ocelli of the foragers of the second group were covered with flat black enamel during training. A 650 W quartz lamp shielded by a heat-absorbing filter was used as the light source. The hive was constructed so that dances occurred only on one side of one frame. The distance of the light from the dance area was varied until the dances of the control foragers—whose ocelli were unpainted—were completely reoriented, while those of the ocelli-painted foragers were not. For 30 min before the experiments began, the light was left on to adapt the bees to its presence. It illuminated the dance area from above, that is, the direction away from gravity. Since that is the direction 'assigned' to the Sun when the dances are performed in the dark, the dance orientations were not affected. At the beginning of the experiment the light was moved to a different angle, and the solution at the ocelli-painted forager station was changed to 2 M peppermint-scented sucrose.

Six experimental stations similar to those designed by Renner²³, and almost identical to the forager station, were set out at the positions shown in Fig. 1. Because many recruits

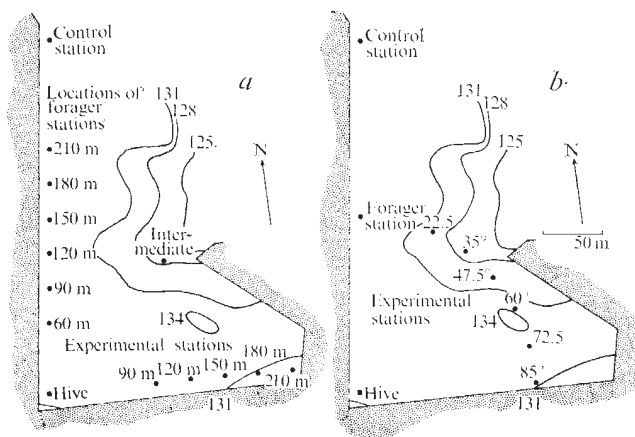


Fig. 1 Experimental array for distance and direction experiments. One group of foragers was trained on 1 M anise-scented sucrose to the control station 300 m to the north. A second group, whose ocelli were covered with paint, were trained in the same direction on 0.5 M orange-scented sucrose. Elevations are given in metres. *a*, Distance experiment, five experimental stations were placed in a line to the east at 30 m intervals. A sixth intermediate station was placed at the location shown, 150 m from the hive. A light in the hive caused recruits to misinterpret the dance of the treated foragers and to search for the food to the east rather than to the north. The forager station was moved every 20 min to a new location. This altered the distribution of recruits at the experimental stations (see Fig. 2). The wind was from 159° at 2 km h⁻¹. *b*, Direction experiment, six experimental stations were set out at 12.5° intervals at 150 m from the hive. Every 30 min the light in the hive was moved, altering the interpretation of the dance direction. This altered the distribution of recruits (see Fig. 3). The wind was from 001° at 10 km h⁻¹.

are hesitant to land unless they can see other bees already there, the 'flower'²⁴ of each station was 'baited' visually with the body of an alcohol-extracted, Sun-dried bee. Each station was 'odour-baited' with the experimental solution and a wire box containing 10 anaesthetised, ocelli-treated foragers captured during training. Entering bees interrupted a beam of red light and the resulting signals from a photocell were recorded on a six-channel event recorder. The stations were filled with CO₂, which anaesthetised the recruited bees while they fed. Recruits arriving at and entering these stations therefore never returned to the hive to dance.

Recruits arriving at the ocelli-treated forager station were captured without the release of odours by a method described previously¹³. Wind speed and direction were automatically recorded while other weather parameters were monitored every 10 min. The dances of both control and ocelli-treated foragers in the hive were measured from video recordings, and the visits of treated foragers to their stations were recorded.

The first experiment was performed to determine whether information about distance is conveyed by the dances. The light was moved 85° to the left of vertical. As a result the dances of the foragers with painted ocelli were 85° to the right (clockwise) of those of the reoriented control foragers, and would therefore be expected to misdirect untreated bees to the right by 85°. The experimental array was located 85° to the right of the forager station (Fig. 1a). The forager station was moved every 20 min to a new distance in the following order; 90 m, 120 m, 150 m, 180 m, 210 m, 150 m, 90 m, and 60 m. Recruits arriving along the experimental array did show a preference for the distance indicated by the dance (Fig. 2).

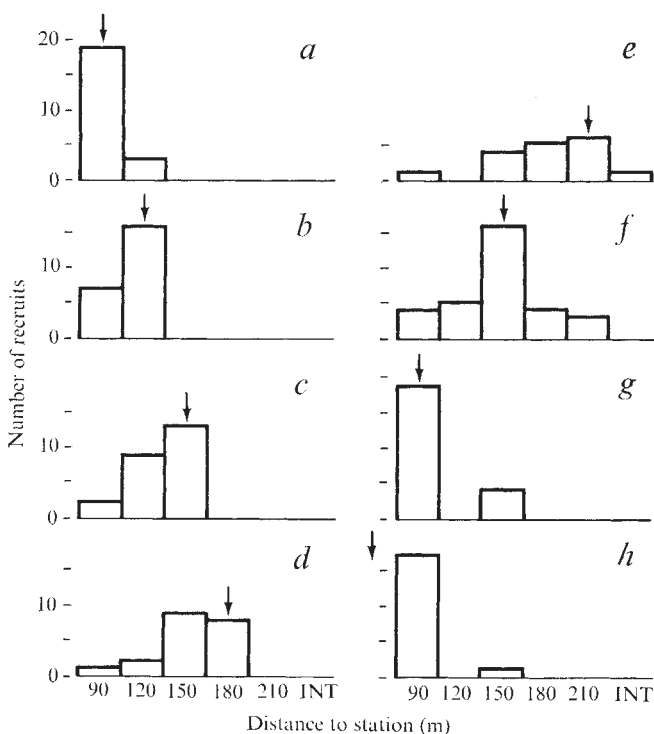


Fig. 2 Results of the distance experiment. The number of recruits captured at various distances from the hive along the experimental array is shown for each location of the forager station. The distance of the forager station is indicated by the arrow (in *h* the distance was 60 m). Each bar graph represents a 20 minute interval.

The second experiment was performed to determine whether directional information is communicated by the dance. The light was moved every 30 min, first 85° to the left of vertical, then to 35° left, and then to 60° left. The control foragers were reoriented by the same amount, so the recruits would be expected to misinterpret the dances of the treated foragers by a

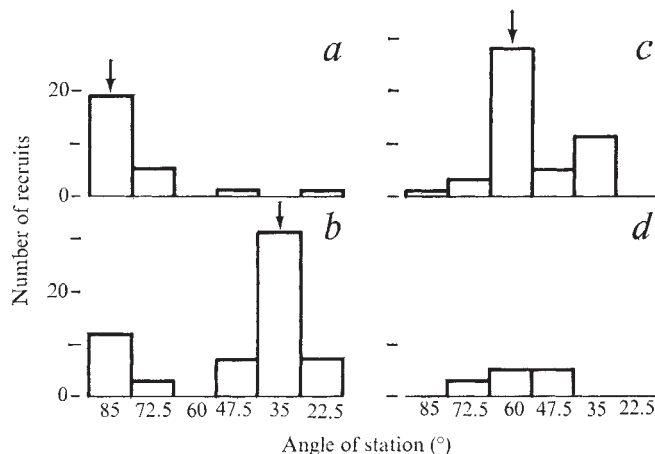


Fig. 3 Results of the direction experiment. The number of recruits captured in various directions from the hive is plotted for several orientations of the light. The arrow indicates the direction which the treated foragers signalled to untreated recruits. The time intervals is 30 min. In *d* the light was moved to the 'up' position and the forager food removed.

corresponding angle to the right. The experimental array was composed of six stations arranged at 12.5° intervals and 150 m from the hive (Fig. 1b). The recruits arriving at the experimental stations did show a preference for the direction expected from the dance (Fig. 3).

Since most of the recruits arrived at locations specified by the dances, but not near the site being frequented by the foragers performing those dances, olfactory information unique to the forager station or its locale does not explain recruitment. Under these conditions the dance clearly communicates abstract distance and direction information which many recruits are subsequently able to use.

Additional details of this technique, these experiments and the results of subsequent experiments will be reported elsewhere. I thank Donald R. Griffin, Peter Marler and Fernando Nottebohm for their criticisms of this manuscript. Financial support was provided, in part, by the Cary Trust, Millbrook, NY.

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Erythropoiesis in a cell population from the early chick blastodisc induced by diffusible product of second cell population

ONSET of rapid haemoglobin formation in the blood islands of the developing chick blastodisc, both *in ovo* and *in vitro*, commences at the stage of the 6- to 7-somite embryo¹⁻⁴ which is normally attained after 28–33 h of incubation⁵. This phase of haemoglobin formation seems^{4,6} to reflect a previous covert differentiation of erythroid-progenitor cells distinct from pre-existing erythropoietic cells which form small quantities of haemoglobin as early as the primitive streak stage of development—after about 20 h incubation⁵.

We⁶ have recently developed procedures for the isolation from dispersed single-cell suspensions of primitive streak and head-fold blastodiscs of two distinct cell populations which form foci of erythropoiesis readily visible to the naked eye when incubated as cell reaggregates. One of these populations was further resolved into two discrete essential subpopulations. Aggregates of either of the individual subpopulations tested singly formed no detectable haemoglobin, whereas aggregates formed by remixing portions of the resolved subpopulations were strongly erythropoietic⁶.

As a first approach to defining the nature of the interaction between these two subpopulations we have now determined that direct contact between them is not required. Rather, one of the subpopulations contains progenitors of cells of the erythropoietic series which differentiate into erythroid cells in response to diffusible product(s) of the complementary subpopulation.

Blastodiscs of the Shaver Starcross No. 288 line of White Leghorn fowl were explanted on to a solid minimal medium³ and those at the primitive streak and head-fold stages of development were selected for these experiments. They were detached from their supporting vitelline membranes and dispersed by incubation with gentle agitation for a total of 20 min in 125 μ l per blastodisc of minimal medium containing 40 units ml⁻¹ of collagenase (Sigma Chemical Company, Type I) and 50 units ml⁻¹ of hyaluronidase (Sigma Chemical Company, Type I). Dispersion was facilitated by gentle flushing with a Pasteur pipette at 10 and 20 min of incubation. Suspensions of single cells were recovered by filtration through one layer each of Nitex No. 73 (Thompson and Co., Montreal) and Nitex No. 53 nylon monofilament cloth.

Volumes (1.2 ml) of suspension containing 1.2×10^7 cells ml⁻¹ were layered on to freshly-prepared, sterile discontinuous density gradients of Ficoll in liquid minimal medium, at room temperature. These consisted of a 1.3 ml upper layer of solution of specific gravity 1.044, a 1.3 ml intermediate layer of specific gravity 1.049, and a 1 ml cushion of specific gravity 1.062. These gradients were immediately centrifuged in Spinco SW 50 rotors at 4,500 r.p.m. for 30 min without braking. Cells retained at the upper (subpopulation A) and lower (subpopulation B) interfaces, respectively, of the upper Ficoll band of the gradient were each transferred to 5 ml of minimal medium—2% foetal calf serum (Gibco, Grand Island, New York) and sedimented at 5,800g for 10 min.

The pellet of subpopulation A was plated with a sterile microspatula on a portion of vitelline membrane laid on the surface of egg homogenate medium⁷, freshly prepared and prewarmed. A layer of dialysis membrane sterilised by autoclaving in distilled water, then equilibrated with Earle's minimal medium containing HEPES buffer (Gibco, Grand Island) and 20% foetal calf serum, was placed over this aggregate and extending beyond it for at least 2 cm on all sides. The pellet of subpopulation B was then plated on the dialysis membrane, and the whole was incubated at 38°C in a moist chamber.

At 4 d the upper aggregate of subpopulation B was

seen to be 'peppered' throughout with foci of erythropoiesis readily visible to the naked eye. By contrast, neither the lower aggregate of subpopulation A nor control aggregates of subpopulation B plated alone on dialysis membrane contained any visible haemoglobin.

This result clearly establishes that the two resolved cell populations play different roles in the differentiation of cells of the erythropoietic line. Moreover, they demonstrate that the progenitors present in subpopulation B differentiate to erythroid cells in response to diffusible material(s) of low molecular weight produced by cells in the complementary subpopulation A.

Miura and Wilt⁸ have previously demonstrated that haemoglobin formation in cells of the mesodermal layer of the primitive chick blastodisc is dependent upon material from cells of the endoderm layer which can diffuse through a Millipore filter. It is therefore tempting to suppose that the essential cells of our subpopulations A and B are derived from the endoderm and mesoderm, respectively. The relationships between the resolved subpopulations and those inferred to be present in the intact blastodisc however remain to be established.

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Preparation of human placental villous surface membrane

THERE is currently considerable interest in the mechanism of protection of the foetus against rejection by the mother¹. Various theories have been put forward to explain this phenomenon, including the existence of an immunologically inert barrier between mother and foetus², and a reduction of immunological reactivity of the mother during pregnancy³. A further possibility is that a hormone such as progesterone binds to the external villous surface of the placenta and masks its antigenicity.

The surface membrane of human chorionic villi represents the effective foeto-maternal junction and therefore a preparation of this membrane would be of value in studying the immunochemistry of the foeto-maternal relationship. We have used a simple saline extraction technique to remove this material from placenta and have studied its composition by electron microscopy. It is likely to contain any antigenic material present as a somewhat similar technique (hypotonic solutions, 0.1–0.8%) was used by Davies⁴ in the preparation of membrane fragments rich in H-2 antigenic activity from cell surfaces of mouse spleen and thymus.

Freshly delivered normal human placentae, whose normality was subsequently confirmed by histology, were used. Preliminary experiments using solutions of varying composition and ionic strength established the optimal conditions. In the procedure finally adopted, a 10–20 g tissue sample is spread out manually and then washed rapidly, first in ice-cold isotonic CaCl_2 solution and then in ice-cold Krebs' physiological saline. This removes much of the blood from the intervillous spaces. The sample is next placed in ice-cold 0.9% NaCl solution and gently agitated at 0° C for 30 min using a magnetic stirrer. During this procedure, the chorionic villi spread out so that their surfaces are well irrigated (Fig. 1a). The saline wash is then poured off and centrifuged for 10 min at 800g to remove fragments of tissue and red blood cells. The resulting cloudy supernatant is centrifuged in a Beckman Spinco at 10,000g for 5 min to remove larger particles and intracellular debris. Then a higher speed run (100,000g for 60 min) yields a pale jelly-like pellet.

For electron microscopic examination, three samples were taken: intact untreated placental tissue, tissue stirred with 0.9% NaCl solution for 30 min and the pellet obtained by centrifuging the NaCl wash at 100,000g. Each sample was placed in 3% glutaraldehyde in buffered solution as quickly as possible.

Examination of untreated tissue kept at 4° C for 40 min and compared with tissue fixed immediately (Fig. 1b), established that no significant deterioration takes place in that time but a striking loss of microvilli was seen in tissue washed with 0.9% NaCl solution (Fig. 1c). It was apparent on scanning several samples of the treated tissue, that many of the chorionic villous surfaces were free or almost free of microvilli and that no other significant changes had occurred, indicating that there had been little or no cellular damage except that due to removal of microvilli. The sedimented pellet (Fig. 1d) contained mainly membrane-bound bodies resembling microvilli. The material removed at 10,000g consisted of larger particles, probably consisting of intracellular fragments from damaged areas.

Preparations of the pellet of microvilli contained a high

percentage of water (85–90%). Protein⁵, carbohydrate⁶, sialic acid⁷ and lipid were also present. The progesterone content of the pellet was relatively high (2–5 μg per g wet weight which is significantly different ($P=0.001$) from that of whole placental tissue (1–2 μg per g wet weight)). Progesterone was estimated by gas-liquid chromatography after purification of the lipid extract by thin-layer chromatography on silica gel.

Work is now in progress on the nature of the association of progesterone with the villous surface of the placenta, as represented by the pellet of microvilli. After solubilisation by 1% sodium deoxycholate, the product is incubated with radioactive (1, 2-³H) progesterone and is then fractionated by ion exchange chromatography, gel filtration and polyacrylamide gel electrophoresis.

We believe that the material removed from placenta by the treatment described consists of microvilli and related membrane fragments, and the evidence for this is the appearance of the detached particles and the fact that the tissue left behind is essentially normal under the electron microscope. The preliminary chemical examination of the pellet is compatible with this view.

The procedure outlined above is simple, rapid and gives a good yield of material. Apart from further studies on the immunology of the foeto-maternal interface, it should also be a useful experimental preparation for general investigations on the biochemistry of cell surface antigens, membrane-bound enzymes and other membrane components. In view of the extremely marked degenerative changes in the trophoblast which are often seen in pre-eclampsia and sometimes in certain other disorders of pregnancy⁸ it may be useful to use this technique in the study of these conditions.

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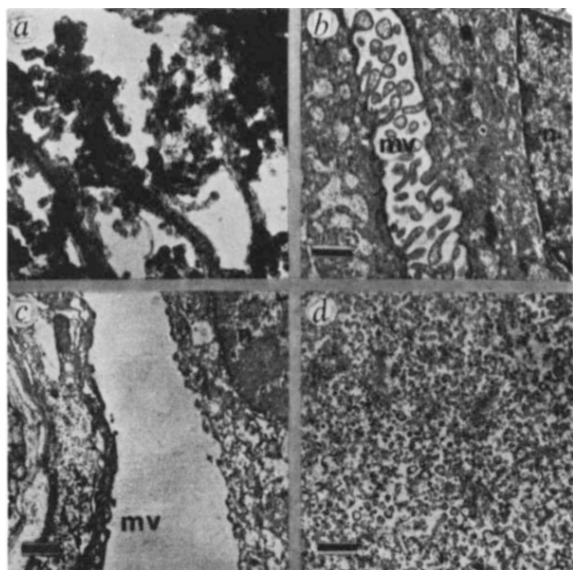


Fig. 1 a, Wet mount of chorionic villi floating in saline ($\times 60$); b, Two adjacent villi before saline treatment; c, after saline treatment; d, 100,000g pellet. Samples for b, c and d were fixed in 2.5% glutaraldehyde in sodium cacodylate buffer, secondarily fixed with 1% osmic acid, dehydrated and then embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate. Bars represent 1 μm in b, c and d. n, nucleus; mv, microvilli.

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The effects of mitomycin C on remyelination in the peripheral nervous system

REMYELINATION within the central nervous system (CNS) is rarely seen in pathological material or in experimentally produced lesions and when it occurs it is a considerably slower process than its counterpart in the peripheral nervous system. It is generally thought that a major factor precluding central remyelination is the failure of oligodendroglia to undergo division^{1,2}. As this hypothesis is difficult to test experimentally

in the CNS, we have attempted to mimic the situation peripherally, and have examined the effects of inhibiting Schwann cell proliferation after peripheral demyelination. Here we report our preliminary results.

This type of experiment is feasible only if the demyelination is not accompanied by destruction or injury of Schwann cell or axon, and a reasonably specific means of blocking cell division can be used in which side effects are minimal. We have shown previously that the intraneural injection of lysophosphatidyl choline (LPC) into the sciatic nerve of the adult mouse will produce a circumscribed demyelinating lesion, without producing detectable damage in either Schwann cell or axons³. Mitomycin C inhibits DNA replication⁴ and has been used successfully to inhibit cell division in early frog embryos⁵.

In the first experimental series, adult mice received intraneural injections (about 2×10^{-4} ml) of a solution of mitomycin C ($400 \mu\text{g ml}^{-1}$ in physiological saline), followed 24 h later by a solution of LPC (10 mg ml^{-1} in physiological saline) (ML24 animals). Animals were killed at various intervals after the LPC injections, and the nerves processed for conventional electron microscopy. Details of the method of injection are given elsewhere³. Controls received an intraneural injection of physiological saline followed by LPC (SL24 animals).

In a second series of mice, $1 \mu\text{Ci ml}^{-1}$ ^3H -thymidine (specific activity about 5 Ci mmol^{-1}) was injected intraneurally at intervals from 1 h to 48 d after the LPC injection; this was chased 45 min later by the intraneural injection of 12 mM thymidine. The animals were killed after 4 h. Controls received saline instead of LPC. A further group of ML24 mice received intraneural injections of ^3H -thymidine at various time intervals 3–20 d after the injection of LPC. Lengths of sciatic nerve were removed and either homogenised in cold 10% TCA, the residues collected on Millipore filters, washed and counted in liquid scintillation medium³, or they were stretched on card, processed as for electron microscopy, 3 μm thick resin sections cut and prepared for light microscope autoradiography.

In a third series of mice, standard intraneural injections of LPC were followed 3 or 7 d later by the intraneural injection of mitomycin C (LM3 and LM7 animals). The animals were killed 20 d after the LPC injections and the nerves processed for conventional electron microscopy.

There was an obvious behavioural difference between the ML24 and SL24 series. ML24 mice exhibited weakness of the hind limb which persisted for up to 14 weeks after the LPC injection; a similar weakness in the SL24 animals disappeared within the first 2 weeks.

Morphologically, the overall consequence of pretreatment with mitomycin C was a retardation of those changes which occur subsequent to LPC-induced demyelination. Thus, in ML24 mice the Schwann cells remained laden with debris for 2–3 weeks, while in the SL24 mice most of this debris was extruded from the axon-associated Schwann cells and taken up sequentially into supernumerary Schwann cells^{6,7} and endoneurial macrophages within the first 10 d. There was a marked

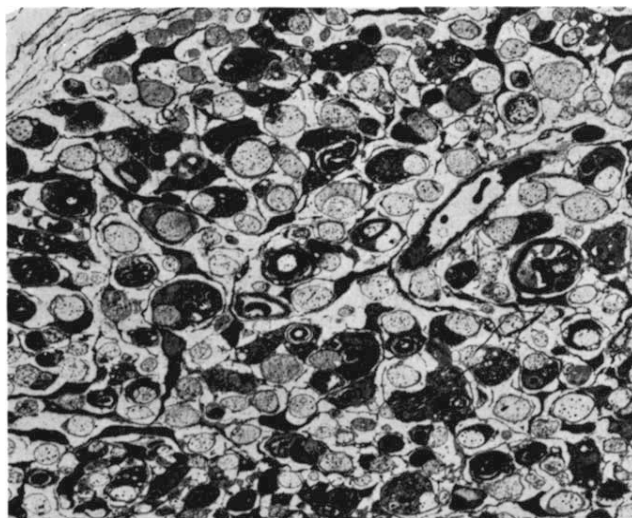


Fig. 1 ML24, 20 d after injection. There has been no remyelination ($\times 980$).

increase in Schwann cell number 6–10 d following demyelination in SL24 mice; there was little increase in Schwann cell number in those animals pretreated with mitomycin C, although in both groups there was an increase in the numbers of haematogenous cells and of fibroblasts. The relative uptake of ^3H -thymidine by SL24 and ML24 nerves indicated that most of this increase in cell number in SL24 mice was due to local cell division (table 1).

In SL24 mice, remyelination was established in at least 80% of the fibres by 20 d, with a concomitant maintenance of the high endoneurial nuclear count, reflecting the persistence of numerous axon-associated and supernumerary Schwann cells (about 15% of the axons were associated with Schwann cell nuclei)⁷. In ML24 mice, however, the Schwann cell nuclear count remained low during the first month after injection (4% of the axons were associated with Schwann cell nuclei, a figure within the limits for normal nerve). Transverse sections of ML24 nerve during this time revealed many axons to be incompletely surrounded by fine processes of Schwann cell cytoplasm, while others (about 25% of the total axonal population in some cases) appeared not to be associated with a Schwann cell. In all cases, the axons, with or without a Schwann cell, were enclosed by a continuous basal lamina. There was no evidence of remyelination (Fig. 1), although in a few Schwann cell/axon units in ML24 mice complex infoldings of Schwann cell plasma membrane were observed, unrelated to the enclosed axon, reminiscent of aberrant mesaxons in neonatal nerve. It is possible that these represented unsuccessful attempts at remyelination.

In ML24 mice many axons exhibited morphological features not normally observed in the axoplasm of normal or remyelinated

Table 1 Uptake of thymidine by LPC-demyelinated nerves with or without mitomycin C pretreatment

4 h thymidine uptake (c.p.m. per nerve)		Mitomycin C-treated (ML24)		Test/Control (% uptake)	
Time after LPC (d)	Controls (SL24)				
6	2377 s.e.m. $\pm$ 449 ($n=3$)	601 s.e.m. $\pm$ 172.8 ($n=6$)		37	
10	832 s.e.m. $\pm$ 295 ($n=3$)	140 s.e.m. $\pm$ 38.5 ($n=5$)		16	
4 h thymidine uptake, differential cell count		Mitomycin C-treated (ML24)			
Time after LPC (d)	Controls (LPC)				
	% labelled Schwann nuclei	Axon-associated Schwann nuclei	% labelled	% labelled Schwann nuclei	Axon-associated Schwann nuclei
5	22 $\pm$ 7.1 ($n=3$)	total	% labelled	total	% labelled
6	29 $\pm$ 6.3 ($n=3$)	129*	48*	3.5 ($n=2$)	—
10	6 $\pm$ 5.3 ($n=3$)	356	9 $\pm$ 8	2.0 ($n=3$)	—
18	2 ($n=1$)	64	3	6.0 ($n=2$)	—
20	3 ($n=2$)	187	4	7.0 ($n=3$)	—

* In two samples, Schwann cells that were axon-associated were also debris-containing, so these data refer to one sample only.

ing axons: large masses of myelin debris, often filling 80% of the axonal cross sectional area (Fig. 2), a proliferation of smooth endoplasmic reticulum, and numerous ribosomes. Intra-axonal lamellar debris was observed most commonly during the first 3 weeks after injection.

During the acute phase (1–20 d) of the response in ML24 mice, the morphology of the fibres 1 cm distal to the lesion was examined routinely in 1 μ m resin sections, and found to be normal in 90% of the mice: however, in 10% some fibres appeared to be undergoing demyelination or Wallerian-type degeneration. In ML24 mice, 200 d after LPC injection, not only had remyelination occurred throughout the site of the lesion, but most fibres distally exhibited short thinly myelinated internodes indicating that they had also undergone demyelination and subsequent remyelination. It is possible that the proliferation of Schwann cells distal to the lesion is a compensatory response, provoked by chronic alterations in the Schwann cell/axon relationship.

In both LM3 and LM7 mice, the progress of the lesion was intermediate between that in the SL24 and ML24 animals. This is compatible with the hypothesis that it is the initial or early cell divisions which are critical in reactivating the myelinating activity of the Schwann cell.

Although in general these experiments support the idea that mitomycin C acts primarily by inhibiting DNA replication and thus cell division, its possible effects at the level of transcription must not be ignored. This aspect of mitomycin C action is being investigated.

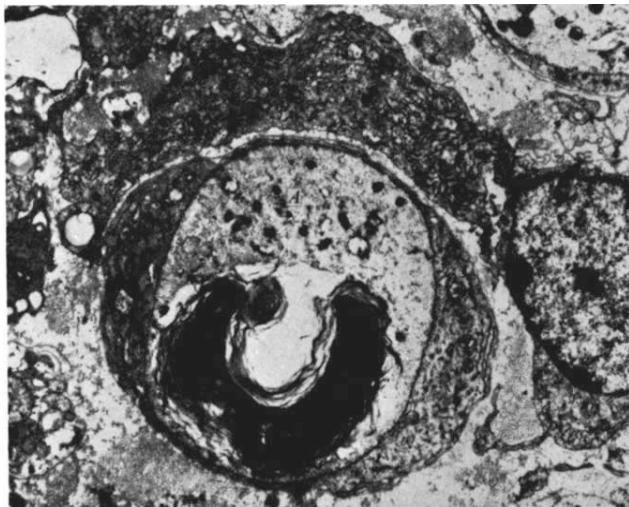


Fig. 2 ML24, 20 d after injection. Note the myelin debris in the axon A ($\times 7,350$).

We conclude that the time course of the cellular response in the peripheral nervous system after the inhibition of satellite cell division at the time of a primary demyelinating episode closely resembles that found in the CNS after demyelination, that is, a slow removal of myelin debris and a failure of early remyelination. This suggests that the converse experiment, a stimulation of oligodendroglial division following primary demyelination in the CNS might lead to a more successful regenerative process centrally.

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Phagocytosis by pigment epithelium of human retinal cones

AUTORADIOGRAPHIC studies of vertebrate retinæ indicate that rod outer segment disks are continuously manufactured at the base of the outer segment^{1,2}. The disks are slowly displaced towards the tip of the outer segment, where they are shed and phagocytosed in groups by the apical processes of pigment epithelial cells^{3,4}. Identical autoradiographic studies indicate that cone disks are not continuously manufactured at the base of the outer segment, leading to the conclusion that they are not phagocytosed⁵. The observation that phagosomes occur within the pigment epithelial cells of the rod-free region of human foveola, however, suggested that outer segment disks from foveal cones are phagocytosed⁶. Are human foveal cones uniquely rod-like in this respect, just as they are rod-like in their form and in their extension to the pigment epithelial surface, or are the disks of all human cones phagocytosed by the pigment epithelium? Our observation of a close association between cone outer segments of cat retina and pigment epithelial cell processes⁷ suggested an answer. As with human extrafoveal cones, those in cat do not reach the pigment epithelial surface; instead, long leaf-like processes extend from the pigment epithelium to the outer segments. These cell processes form a multilaminar sheath around the external one-third of the outer segment. If these processes engage in phagocytosis, phagosomes containing groups of cone disks should be observed within their cytoplasm and in good sections there should be very little chance of confusing them with phagocytosed rod disks. In human retinæ, where cones are more plentiful than in the cat, the extrafoveal cone outer segments are also closely associated with long pigment epithelial processes^{8–11}. We report here that these processes phagocytose groups of disks from the cone outer segments.

The retina used for this study was obtained from the left eye of a 5-yr-old patient with an orbital rhabdomyosarcoma. The eye did not show any pathological changes. Immediately after surgical removal, the eye was transected into anterior and posterior segments and placed in fixative. The posterior segment was then dissected to isolate the macular region (Fig. 1). Sections were cut (1 μ m) for orientation purposes, stained with basic fuchsin and methylene blue and examined with a Zeiss photomicroscope. Ultrathin sections were cut from the aligned specimen, stained with uranyl acetate in H₂O and lead citrate, and examined in a Siemens-Elmiskop 1A electron microscope.

Human extrafoveal cones are readily distinguished from the surrounding rods; cone outer segments are shorter than rod outer segments while cone inner segments are much wider than rod inner segments. Cones are also identified by long processes which reach their outer segments from the pigment epithelium. Some of these processes adjoin the cone outer segment for at least one-half of its length.

The basic features of the cone and its relation to the pigment epithelium are seen by light microscopy (Fig. 1). The pigment

epithelial processes extend from the epithelial surface to occupy the space above the cone outer segment tips. Oval melanin granules occur within the processes close to the epithelial surface. There are also two irregular inclusions within the epithelial processes close to the outer segment of the cone on the left and there is at least one irregular inclusion in the processes of the cone on the right (arrows in Fig. 1). These inclusions stain like the outer segment but somewhat more intensely and may be phagosomes. Their identification as such can be made only with the electron microscope.

Of the cones surveyed by electron microscopy, at least one-third showed phagosomes within the epithelial processes. There is usually more than one phagosome within the processes that associate with a single cone, and as many as five have been identified. The cone illustrated in Fig. 2a has two phagosomes (arrows). They can be distinguished readily from melanin granules, which are usually oval and do not have a disk-like substructure. Each phagosome consists of a group of cone disks still enclosed within the cell membrane of the outer segment (Fig. 2b). The portion of cone outer segment is itself surrounded by the membrane of the epithelial processes (Fig. 2b, arrows). The disks of this phagosome (Fig. 2b) have maintained their original orientation while the disks of the other phagosome have rotated 90°. The phagocytosed disks stain more deeply than the disks of the intact outer segment.

The processes containing the phagosomes are continuous with the processes that surround the outer segment along its outer half (Fig. 2a and c). Here, the processes are directly involved in phagocytosing the tip of the outer segment. A single process may bifurcate so that it surrounds the tip (Fig. 2c, arrow). Figure 2d shows the tip of another outer segment where a pigment epithelial process has extruded a pseudopod (arrow) which traverses the outer segment and isolates a group of 40 disks from the remainder of the outer segment. Other stages in the phagocytic process also have been seen.

Phagocytosis of portions of human cone outer segments is similar to that found in human and Rhesus monkey rod outer segments. For both classes of photoreceptor the pigment epithelial processes intermittently surround and then engulf a group of disks from the tip of the photoreceptor^{12,13}. This suggests that human cone disks also must be continuously renewed, although the mechanism of renewal may differ from that of the rod. This conclusion is supported by the observation

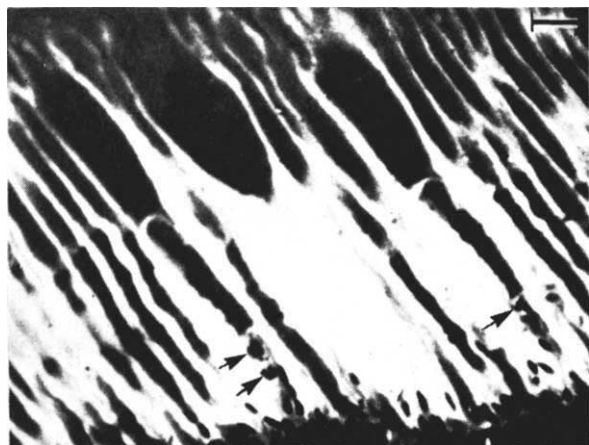


Fig. 1 Light micrograph of two cones. Fixation took place in 3% glutaraldehyde-paraformaldehyde, 0.2 M Na cacodylate buffer with the addition of 1% sucrose, CaCl_2 and KCl, pH 7.4 for 2–3 h at room temperature. This was followed by post fixation in 2% osmium tetroxide in Na veronal acetate buffer with the addition of sucrose for 2 h and then by block staining in 2% uranyl acetate with Na H maleate buffer for 2 h (dark). The tissue was dehydrated in graded acetone and embedded in Araldite. The arrows point to inclusions within the pigment epithelial processes, which are presumed to be phagosomes. Bar equals 5.0 μm .



Fig. 2 a, Electron micrograph montage of a cone. Slender pigment epithelial processes, containing two phagosomes (arrows), extend to the outer segment tip. This section is somewhat atypical in that the processes are fewer in number and thinner than in the average section. b, Enlargement of phagosome closest to outer segment tip. Pigment epithelial cell membrane (arrows) surrounds a group of 62 disks. c, Enlargement of outer segment tip showing bifurcation of a pigment epithelial process (arrow). d, Outer segment tip of a different cone. A pseudopod from a pigment epithelial process extends across the outer segment isolating the 40 terminal disks. Bars equal 0.5 μm (a, b, c); 0.25 μm (d).

that Rhesus monkey cone outer segments regenerate after having been damaged by separation from the pigment epithelium¹⁴.

Finally, that continuous renewal of cone disks may be general in mammals is suggested by a recent description of disk shedding by the cone-like photoreceptors of diurnal tree squirrels¹⁵.

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Antigenic specificity of retinal pigment epithelium and non-immunological involvement in retinal dystrophy

ORGAN specific antigens have been demonstrated in the retina proper and particular attention has been paid to their relation to eye disease^{1–7}. The antigenicity of the photoreceptors has

also been demonstrated⁸ and its implication in the pathological process of retinal dystrophy in experimental animals and in man has been lately under evaluation^{9–10}. In the case of the retinal pigment epithelium (RPE), however, no conclusive evidence of its antigenicity or immunological involvement in ocular disease has so far been produced. Nevertheless this layer fulfils an important physiological role^{11–13} and its primary involvement in retinal dystrophy has been emphasised^{14–17}.

This study was carried out to determine the antigenicity of the RPE and to determine whether any specific autoallergic reaction to RPE may be found in retinal dystrophy in rats. RPE obtained from normal albino rabbits was grown as cellular monolayers in tissue culture; the tissue was freeze-dried and stored. A Dunkin Hartley adult guinea pig was immunised by six injections of 4 mg of the freeze-dried material given subcutaneously at weekly intervals together with Freund's complete adjuvant. The animal was killed at the end of the experiment and the serum was stored at -20°C until used. Several Cryostat sections of four normal eyes from albino rabbits and several primary cultures of RPE from 12 normal eyes of albino rabbits and the same number of normal eyes from Wistar albino rats, were studied by indirect immunofluorescence technique, using fluorescein-labelled anti-guinea pig serum. Cryostat sections and primary cultures treated first with normal guinea pig serum and then with fluorescein-labelled anti-guinea pig serum were used as controls. Antibodies to RPE were present in the sensitised guinea pig serum (Fig. 1), which reacted intensely with cultured rabbit RPE and somewhat weakly with cultured rat RPE; both cytoplasmic and membranous fluorescence were observed suggesting that the antigens are located on the surface membrane as well as intracellularly. Whether or not these represent two distinct antigen systems requires further study. In whole cryostat

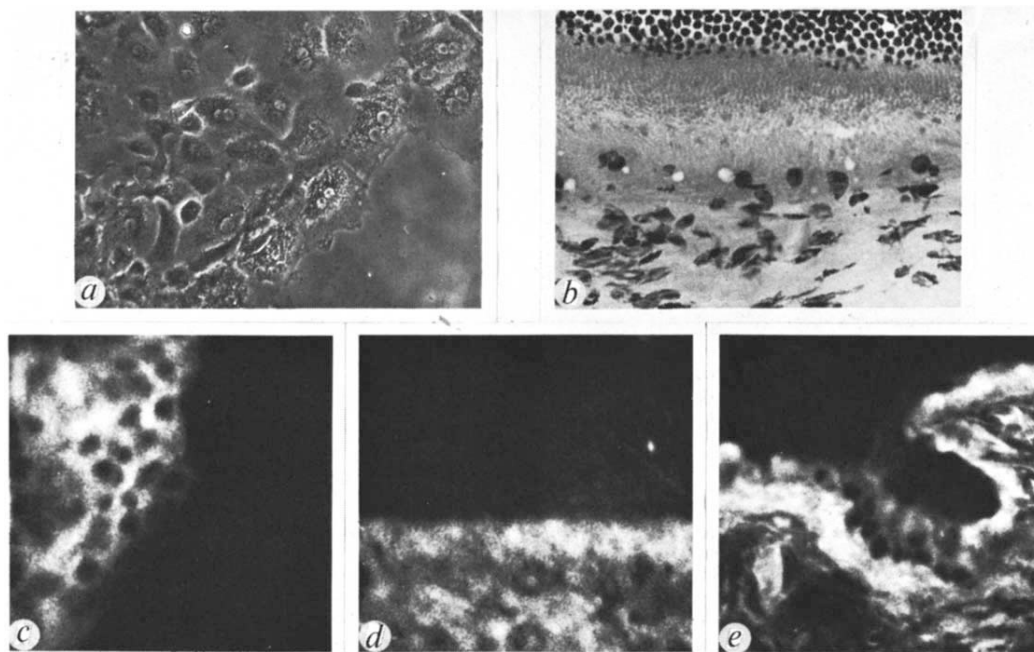


Fig. 1 Indirect immunofluorescence study of Cryostat ocular sections and RPE cultures from albino rabbits. The sections of sensory retina, RPE and choroid were cut in a tangential oblique plane; ciliary processes were sectioned anteroposteriorly. Primary explants of rabbit RPE, grown on glass, were cultured in medium 199 (Wellcome) and heat-inactivated rabbit serum (30%) to which fresh glutamine (Bio Cult) was added (300 mg per 1000 ml). Cultures on their glass coverslips were thoroughly rinsed in warm (37°C) balanced salt solution. Both Cryostat sections and primary cultures were fixed in ether/acetone before sequential incubation with sensitised or with normal guinea pig serum and fluorescein labelled anti-guinea pig serum (Wellcome); they were incubated at room temperature for 30 min and washed in a bath of phosphate buffer saline (pH 7.6) for 30 and 45 min. respectively. All sera were diluted 1 : 10. *a*, 3 day-old primary culture of rabbit RPE (phase contrast, $\times 82.5$); *b*, cryostat section of retina, RPE and choroid (haematoxylin and eosin $\times 72.5$); *c*, indirect fluorescence localisation in 3-day old primary culture of rabbit RPE ($\times 99$); *d*, cryostat section of retina, RPE and choroid. Note the intensity of RPE fluorescence outlined as an approximately horizontal band between the non-fluorescent retina and the underlying fluorescent choroid ($\times 198$); *e*, cryostat section of ciliary processes. Note the convoluted fluorescent layer of the pigmented epithelium upon the surface of which the non-pigmented ciliary epithelium can be observed with a background negative fluorescence. The stroma presents a weak positivity ($\times 198$).

sections of rabbit eyes no fluorescence was seen in the sensory retina including photoreceptors, thus further suggesting that the RPE which develops from the outer layer of the secondary optic vesicle is antigenically distinct from the sensory retina, especially the photoreceptors, which develop from the inner layer of the secondary optic vesicle.

A positive fluorescence was, however, observed in the RPE and pigmented layer of the ciliary epithelium and also in the extravascular choroidal tissue. As the pigmented epithelium of the ciliary body is the forward continuation of the RPE it is understandable why this tissue cross-reacted with the anti-RPE serum; the fluorescence of the choroid remains unexplained but it suggests that extravascular choroidal tissue has some antigenic material in common with the RPE. As growth patterns of cultured RPE are quite different from those of cultured choroidal mesenchyme and as extreme care was taken to obtain pure RPE for tissue culture it seems highly unlikely that the materials we used for sensitising guinea pigs were contaminated with choroidal tissue, but it is a possibility which is difficult to rule out.

To investigate whether autoantibodies to RPE could be demonstrated in cases of rat retinal dystrophy, flat preparations of RPE were obtained from Wistar albino rats and inbred PETH dystrophic rats of comparable age groups. Serum was obtained from two groups of inbred PETH dystrophic rats: first, animals of approximately 3 weeks of age showing early retinal degeneration and, second, animals of 1.5–2 months of age showing marked retinal degeneration. Indirect immunofluorescence study using fluorescein-labelled anti-rat immunoglobulin serum failed to demonstrate any antibodies against RPE in dystrophic animals.

For the first time it has been possible to demonstrate the antigenicity of the RPE and it seems that the antigen present within it is quite distinct from those present in the visual cells of the retina, but no evidence was obtained that this plays a role in retinal dystrophy of the rat.

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X-ray dose response for mutation to fructose utilisation in cultured diploid human fibroblasts

THE majority of studies relating to somatic cell mutation in cultured mammalian cells have been performed with established (heteronuclear) mammalian cells². There are important karyotypic and metabolic differences, however, between cultured heteronuclear cells and mammalian cells *in vivo* and mutation in cultured diploid (homonuclear) cells may be more relevant to the *in vivo* situation. Mutation, in the broadest sense of the term¹, has been studied in cultured diploid cells^{2–9} and there is evidence, from the work of Albertini and DeMars² with 8-azaguanine resistant (Ag^r) variants of diploid human fibroblasts, that it may be possible to quantify radiation-induced mutation in diploid mammalian cells. Here we report that the utilisation of fructose, in place of glucose, as the major carbon and energy source, was used as a phenotypic character for mutation studies with a culture of diploid human fibroblasts. Although not yet fully understood, the fructose-utilisation mutation system does circumvent some of the problems of mutation expression time associated with the selection of Ag^r variants of cultured mammalian cells.

Early passage HF10 diploid human fibroblasts (6–25 generations *in vitro*) grew to confluency when the hexoses D(+) galactose or D(+) mannose replaced D(+) glucose as the major carbon and energy source in Eagles minimal essential medium (MEM) containing 10% dialysed foetal calf serum (MEMFCS). The hexose, β D(–) fructose, did not replace

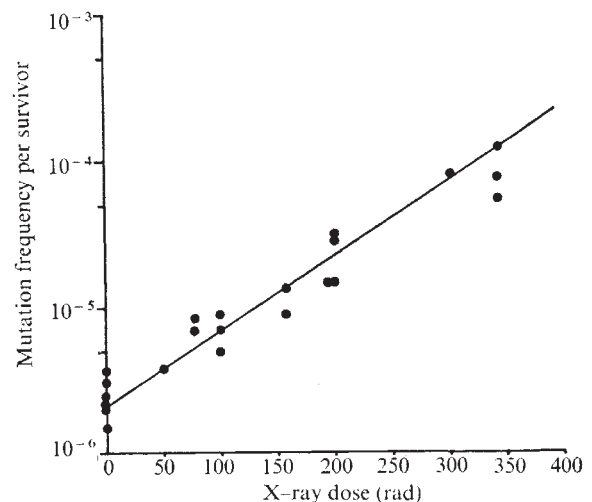


Fig. 1 X-ray dose-response for induction of fructose-utilising variants of 6-25 generation HF10 diploid human fibroblasts. Each point represents between ten and twenty variant clones scored. HF10 diploid human fibroblast cultures were initiated from ♂ embryonic limb tissue and maintained in monolayer culture at 37°C using methods previously described¹². Cells for mutation induction experiments were trypsinised from culture vessels during the exponential phase of growth and resuspended in hexose-free growth medium (Eagles minimal essential medium (MEM) plus 10% foetal calf serum, Flow Laboratories, Irvine, Scotland). Cells were irradiated in suspension with varying doses of 250 kV X rays, dose rates varying between 50 and 250 rad min⁻¹. Immediately after irradiation 2 × 10⁵ cells were added to each 9 cm tissue culture grade Petri dish (Sterilin, Richmond, Surrey, England) which contained 10 ml glucose-free MEM containing 1 mg ml⁻¹ β D(–) fructose (Sigma, Kingston, Surrey, England) plus 10% dialysed foetal calf serum. The serum batch used in these experiments was dialysed for 4 h at 4°C against 100 volumes of 0.85% NaCl. Petri dishes were incubated at 37°C in CO₂ enriched air for 14 d and variant clones arising in the fructose medium were stained for 1 h with 0.25% Azur A stain (Searle Diagnostic, High Wycombe, England). Mutation frequencies after X-irradiation were calculated with respect to the fraction of cells surviving each dose and the dose-response curve drawn by eye through the data points.

Table 1 Distribution of clones utilising fructose amongst Petri dishes containing irradiated and non-irradiated HF10 cells.

Dose (rad)	No. of Petri dishes*	Total cells surviving radiation	Total Fruc ⁺ clones	Number distribution of Fruc ⁺ clones†					Mutation frequency per survivor‡
				0	1	2	3	4	
0	175	3.5×10^7	109	97 (94)	56 (58)	13 (18)	9 (4)	0 (0.5)	3.1×10^{-6}
100	40	2.4×10^6	17	29 (26)	6 (11)	4 (2)	1 (0.3)	0 (0.03)	7.1×10^{-6}
200	39	7.8×10^5	22	23 (22)	11 (12)	4 (3)	1 (0.6)	0 (0.1)	2.8×10^{-5}
343	40	2.8×10^5	21	24 (24)	12 (12)	3 (3)	1 (0.6)	0 (0.7)	7.5×10^{-5}

* 2×10^5 cells per 9 cm plastic Petri dish.†Figures in parentheses are expected Poisson distributions of total Fruc⁺ clones.‡Minimum estimate not corrected for efficiency of recovery of clones².

glucose as a carbon and energy source and HF10 cells degenerated in fructose MEMFCS at the same rate as they did in hexose-free MEMFCS. Limited growth of HF10 cells occurred in hexose-free MEMFCS unless the foetal calf serum (FCS) added to the growth medium was dialysed to reduce the concentration of glucose in the serum.

With the optimum number of HF10 cells (1×10^5 – 2×10^5) in 9 cm tissue culture grade plastic Petri dishes containing 10 ml fructose MEMFCS, fructose-utilising (Fruc⁺) clones arose at a spontaneous frequency of approximately 10^{-6} . As no clones were seen when fructose was omitted from the medium, other medium or serum components are of themselves insufficient. This observation contrasts with the work of Varshaver *et al.*⁶ who found that human diploid fibroblasts selected by their ability to grow in a fructose medium were probably dependent for growth on other medium or serum components.

Three Fruc⁺ clones isolated from HF10 cultures were karyotypically normal, had stable phenotypes during ten generations *in vitro* and were able to grow to confluency with glucose or fructose as a major carbon and energy source. Two Fruc⁺ variants that were tested were also biochemically distinguishable from the parental strain HF10 cells; both Fruc⁺ clones exhibited a marked increase in the rate of uptake of ¹⁴C-fructose and ¹⁴C-glucose (R.C., and W.K.M., unpublished). Preliminary mutation fluctuation tests^{4,7,9} showed that Fruc⁺ phenotypes arose in a random fashion in parallel unselected HF10 cultures, independent of the selective growth conditions. These observations alone, however, are insufficient to enable us to comment upon the genetic basis of Fruc⁺ variants of diploid human fibroblasts.

Preliminary experiments with X-irradiated cultures of HF10 showed that the frequency of the Fruc⁺ phenotype amongst survivors was greater than that observed in unirradiated controls. Mutation expression time experiments were performed, with varying periods of post-irradiation growth in non-selective medium (glucose MEMFCS) before transfer of the cells to fructose MEMFCS. The maximum yield of radiation-induced Fruc⁺ clones was found when the cells were transferred to fructose medium immediately after irradiation, showing that the Fruc⁺ phenotype was fully expressed at zero expression time¹⁰.

The X-ray dose-response curve for the induction of the Fruc⁺ phenotype, when irradiated cells were plated in fructose medium immediately after irradiation, is shown in Fig. 1. The mutant frequency per surviving cell was cumulative and increased approximately exponentially with dose from 2.0×10^{-6} at zero dose to 1.0×10^{-4} at 350 rad. There was no absolute increase, however, in the number of mutant clones per Petri dish (Table 1), and this ratio showed no significant change with dose. This result could indicate a chance coincidence, that is, that the rates of cellular mutation and inactivation were approximately the same over this dose range. Another possible explanation of the findings is that the Fruc⁺ variants were more radio-resistant than the parental strain, and that the radiation, rather than the growth medium, was selecting the variant clones. This seems unlikely because cell cultures grown from two of the Fruc⁺ variants had X-ray survival curves similar

to that of an HF10 parental culture of comparable generation number¹¹ (Fig. 2). A further possibility that the Fruc⁺ variants detected in irradiated cultures were not induced by the radiation but arose spontaneously during the growth and division of cells surviving the radiation is also unlikely as the cells were plated into selective medium immediately after irradiation, thus allowing no time for spontaneous Fruc⁺ variants to arise from the growth and division of non-variant surviving cells. The preliminary conclusion is therefore that Fruc⁺ variants were induced by ionising radiation and that the dose-response relationship was approximately exponential.

Albertini and DeMars² found that, like the Fruc⁺ variants reported here, Ag⁺ variants of diploid human fibroblasts also did not show an absolute increase in number after X-irradiation but did show a dose-dependent increase in mutant frequency per surviving cell. An essentially similar result has been obtained independently in this laboratory with Ag⁺ variants of HF10 cultures but because a post-irradiation growth period of ~3 d in non-selective medium was necessary for optimum recovery of Ag⁺ clones, the interpretation of any dose-response curve must be complicated by the problem of spontaneous mutation of survivors. The Fruc⁺ variants, with an apparent zero mutation expression time, may therefore provide a technically more straightforward mutation system.

If the failure to demonstrate an absolute increase in mutant number with dose reflects the relatively poor mutagenicity of X rays in diploid human fibroblasts, N-methyl-N-nitro-N-nitrosoguanidine (MNNG), which has been shown to be a more potent mutagen than X rays in cultured heteronuclear mammalian cells¹³, should give an absolute increase in mutant number with dose. Preliminary experiments with MNNG suggest that this hypothesis is correct and an absolute increase in mutant number with dose has been observed for both Ag⁺ variants² and Fruc⁺ variants (R.C., and W.K.M., unpublished) of diploid human fibroblasts.

Although some doubt must remain over mutation experiments that do not demonstrate an absolute increase in mutant

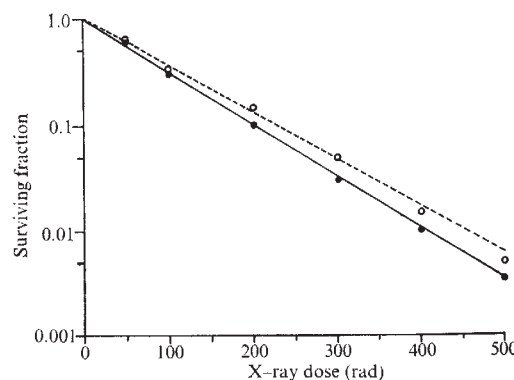


Fig. 2 X-ray survival curves of 25 generation cultures of, ●, HF10 parental strain and, ○, a derivative fructose-utilising variant, Fruc-2. X-ray survival data were obtained using the 'feeder cell' technique previously described¹¹.

Table 2 Forward mutation rates in mouse germ cells *in vivo* and diploid human fibroblasts *in vitro* after single exposures to X rays at dose rates 50–250 rad min⁻¹

Mutation system	Mean induced mutation rate per locus per rad	Dose range	Reference
Mouse spermatogonia (mean of seven loci)	2.64×10^{-7}	0–300 rad	14*
Mouse oocytes (mean of seven loci)	1.08×10^{-7}	0–50 rad	14*
	3.77×10^{-7}	0–200 rad	
	5.41×10^{-7}	0–400 rad	
Diploid human fibroblasts (Ag ^r)	$1.13 \times 10^{-8\dagger}$	0–75 rad	2
	$7.49 \times 10^{-8\dagger}$	0–125 rad	
	$6.87 \times 10^{-8\dagger}$	0–150 rad	
	$2.16 \times 10^{-7\dagger}$	0–250 rad	
Diploid human fibroblasts (Fruc ⁺)	$4.00 \times 10^{-8\dagger}$	0–100 rad	Present work
	$1.24 \times 10^{-7\dagger}$	0–200 rad	
	$2.10 \times 10^{-7\dagger}$	0–343 rad	

*Data also available on mutation induction in mouse germ cells at lower dose rates.

†Calculated as mutation rate per survivor after correction for efficiency of recovery of clones.

‡Calculated as mutation rate per survivor with no correction for efficiency of recovery of clones.

number with dose, the results of Albertini and DeMars² and those reported here suggest that the Ag^r and Fruc⁺ mutation systems may be useful for quantitative studies of mutation in cultured diploid human fibroblasts. It is also possible that the cumulative X-ray dose response observed with both systems is a true reflection of radiation induced mutation in cultured diploid human fibroblasts. A more complete study of the genetic basis of somatic cell variants and the phenotypic expression of induced mutation with these and other mutation systems⁵, however, is required before dose-response curves for mutation can be interpreted with confidence.

With these experimental limitations in mind, it may be useful to compare X-ray mutation rates obtained *in vitro* in cultured diploid human fibroblasts and *in vivo* in mouse germ cells (Table 2). For dose rates between 50 and 250 rad min⁻¹ there are insufficient data to determine the shape of the dose-response curve for mutation in mouse spermatogonia, although there is evidence that mutation induction per rad is non-linear¹⁴. In mouse oocytes¹⁴ and with both mutation systems in diploid human fibroblasts there is fairly clear evidence of a non-linear cumulative dose-response. Mutation rates for Ag^r and Fruc⁺ variants of diploid human fibroblasts seem to be closely similar. For experiments at comparable dose rates and doses of 200–300 rad, the *in vitro* mutation rates in diploid human fibroblasts are also similar to *in vivo* mutation rates in both mouse spermatogonia and mouse oocytes. Although such numerical similarities could be misleading, it is hoped that further studies on radiation-induced mutation in cultured diploid somatic cells may help to elucidate the underlying cellular mechanisms involved in mutagenesis of normal mammalian cells *in vivo*.

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TRH: a possible mediator of thermoregulation

FELDBERG and Myers¹ have reported that intraventricular injections of amines produce changes in the rectal temperature of unanaesthetised cats. They noted that more than 50 µg noradrenaline produced temporary hypothermia whilst 200 µg 5-hydroxytryptamine (5HT) induced a sustained hyperthermia. These results, coupled with earlier findings that these amines were concentrated in the hypothalamus^{2,3}, led them to speculate that the regulation of body temperature in the cat may be mediated by the release of noradrenaline and 5HT within the hypothalamus.

Observations by other workers have raised the possibility that the ratio of cations in the hypothalamus⁴ or the release of prostaglandin E₁⁵ from the same structure may also be important for the chemical mediation of body temperature. This report concerns the effects produced when small quantities of releasing hormones, peptides also derived from hypothalamic tissue, were injected directly into the cerebral ventricles of conscious cats.

Sterile solutions of thyrotropin releasing hormone (TRH), luteinising hormone releasing hormone (LRH) and melanocyte stimulating hormone release-inhibiting factor (MIF) were injected into conscious cats in 0.2 ml artificial cerebrospinal fluid by a cannula previously implanted in the lateral ventricle. LRH and MIF were without effect on body temperature at doses up to 5 µg. In contrast, similar doses of TRH produced a dose-related hypothermia (Fig. 1). Although the minimal effective hypothermic dose varied between individual animals, in some cats as little as 3 ng TRH produced 0.5° C fall in body temperature. At doses greater than 0.5 µg, TRH (in common with hypothermic doses of noradrenaline and calcium) produced a sedative or quieting effect. In addition, all three peptides frequently produced defaecation and urination whilst TRH alone induced profuse salivation in some animals.

The doses of TRH needed to induce hypothermia compare favourably with the calculated TRH content of the hypothalamus^{6,7} and stand in contrast to the fact that the quantity of noradrenaline needed to provoke hypothermia (50 µg) greatly exceeds the endogenous hypothalamic concentration of that amine^{2,8}. Indeed, TRH seems to be the most potent hypothermic agent yet reported (Fig. 2) comparable in its potency to the hyperthermic activity reported by Milton and Wendland⁹ for prostaglandin E₁.

The comparison between noradrenaline and TRH is also interesting from other points of view. Noradrenaline has been reported to stimulate the synthetase enzyme responsible for TRH production whilst 5HT seems to inhibit this same enzyme⁹. Calcium ions, known to be critically involved in the release of noradrenaline from nerve endings^{10,11}, themselves produced hypothermia⁴ whilst imipramine, an antidepressant drug which inhibits the reuptake of noradrenaline into nerve endings, also produces

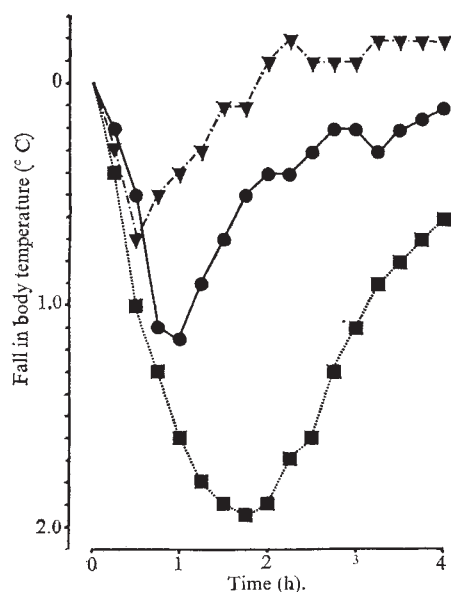


Fig. 1 Hypothermia produced in individual cats by the intravenous injection of, ∇ , 0.05 μg ; $\bullet$, 0.5 μg ; $\blacksquare$, 5 μg TRH. Drug injected at time zero.

hypothermia in cats¹². Thus, it is possible to explain the hypothermia produced by all of these agents in terms of the TRH-induced hypothermia reported here.

Cooper *et al.*¹³ noticed a species difference to the effects of amines on body temperature when they found that intraventricular noradrenaline produced hyperthermia in rabbits. This observation has been confirmed by Jacob¹⁴ and is in keeping with the observation that intraventricular desipramine also produces hyperthermia in this species¹². Preliminary observations in rabbits have shown that doses of 0.5 or 1.0 μg TRH injected intraventricularly produce a hyperthermia of 0.6–1.0°C.

There is speculation amongst psychiatrists as to whether TRH is an effective antidepressant agent^{15,16,17}. My results are consistent with the suggestion that TRH may possess antidepressant activity and may add a new dimension to

the amine theory of affective disorders¹⁸ in that the amines may act indirectly by their effects on TRH production. Whether this is proven or not, the present results certainly indicate that TRH needs careful evaluation as a possible modulator of mammalian thermoregulation.

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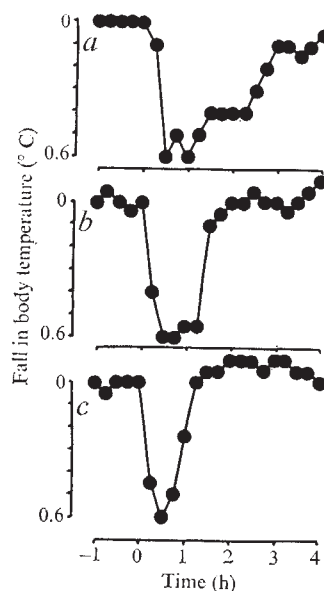


Fig. 2 Hypothermia produced in individual cats by a, artificial CSF containing 20 mM excess Ca^{2+} ; b, 50 μg noradrenaline; c, 10 ng TRH. All drugs were injected at time zero.

A merozoite vaccine effective against *Plasmodium knowlesi* malaria

Plasmodium knowlesi malaria is uniformly fatal in rhesus monkeys¹. If infections are initially controlled with drug therapy, however, a degree of immunity can be induced associated with periodic relapse and low grade infection after repeated challenge. In such resistant monkeys each recurring infection is attributable to a distinct parasite variant recognisable by the schizont-infected cell agglutination (SICA) test². Antigenic variation therefore seems to be an important mechanism determining chronicity of infection³ and complicates any attempt to achieve effective immunoprophylaxis⁴. Rhesus monkeys have been vaccinated with formol-treated^{5,6} or freeze-thawed⁷ erythrocytic schizonts of *P. knowlesi* or fractions of these parasites^{8,9} in complete (FCA) or incomplete (FIA) Freund's adjuvant. Such vaccination reduced mortality to about 50% after challenge with the homologous variant; in surviving monkeys parasitaemia reached 3–10%, but parasites were completely eliminated after about 2 weeks and some animals were then resistant to heterologous challenge⁷. When the first challenge variant was known to differ from that used for immunisation, all monkeys suffered fatal infections⁷. The protection achieved always required the use of complete adjuvant, but this alone was inactive.

Table 1 Infection of normal rhesus monkeys with 10^4 *P. knowlesi* parasites (control infections for challenge of immunised animals)

Parasite* (variant)	Patent (day)	Maximum parasitaemia† (per 10^4 erythrocytes)	Death (day)	Drug cure (day)
W(1)	4	1800	8	
W(1)	3	670	10	
W(1)	3	320		5
W(3)	5	3000	9	
W(3)	3	2000	7	
W(42)	3	130		6
W(12)	4	1100	8	
N(1)	4	500		6

*W, Walter Reed strain; N, Mill-Hill ('Nuri') strain of *P. knowlesi*. Numbers denote distinct variants differentiated by the SICA test^{2,17}.

†Parasitaemia on the day before death or drug cure.

Studies on the mechanism of acquired malarial immunity *in vivo*¹⁰ and *in vitro*¹¹ indicated that protective antibody does not affect intracellular parasites, but interrupts the cycle of development at the stage when merozoites are released into the plasma. In addition, immune serum agglutinated free merozoites¹² and prevented their attachment to receptors present on the red cells of susceptible species¹³. These findings suggested an important role for merozoite antigens in specific malarial immunity and prompted development of an *in vitro* method for isolating extracellular blood stage merozoites¹⁴. Here we describe the use of these preparations for vaccination of rhesus monkeys against *P. knowlesi* malaria.

Monkeys (*Macaca mulatta*) were of either sex and weighed about 3 kg. Parasites were originally obtained from sources indicated in Table 1. Variants of *P. knowlesi* were differentiated by the SICA test². Merozoite preparations (W1 variant) were isolated as described¹⁴ except that phytohaemagglutinin was used in place of antiserum to agglutinate parasitised and normal red cells. The yield of merozoites was approximately 2×10^{10} per ml of cultured erythrocytic schizonts and contamination with parasitised red cells was less than 0.1%. Two monkeys were immunised three times during 7 weeks with 1.3×10^8 mature schizonts (W1 variant) in FCA as controls for the schizont contamination of the merozoite vaccine. Freshly isolated merozoites suspended in 1 ml medium 199 (Burroughs-Wellcome) were emulsified with equal volumes of FCA or FIA and administered by intramuscular injection to six monkeys as shown

in Table 2. Immunisation never produced a detectable infection. Immunised animals were challenged by intravenous injection of 10^4 or 10^5 immature schizonts of known strain and variant specificity on days shown in Table 2. Thick and thin blood films were examined five or more times weekly from the time of challenge. Equivalent challenge infections were administered simultaneously to normal rhesus and all suffered progressive infections. Five out of eight of the control animals died within 7–10 d and three others were cured on day 5 or 6 when the level of parasitaemia indicated a fatal outcome.

Three merozoite immunised animals were challenged first with the same variant (W1) as used for immunisation. No infection was detectable in two of these monkeys (Mz 12, 10, Table 2) and subinoculation of 5 ml of blood from one into a normal rhesus on the 11th d after challenge revealed no viable parasites (Mz 10, Table 2). One monkey (Mz 16, Table 2) challenged with W1 variant developed parasitaemia for 6 d with a maximum of 0.04%. A schizont-immunised rhesus challenged with the homologous variant showed parasitaemia for 11 d with a peak of 1.3% (cf. ref. 7). Three other merozoite immunised animals were challenged first with a heterologous variant (W3) of *P. knowlesi*. All showed parasitaemia which lasted for 8–12 d and did not exceed 0.07–1.5% in the individual monkeys (Mz 14, 15 and 17, Table 2). A schizont-immunised animal challenged first with the W3 variant developed a fatal infection indistinguishable from that in unimmunised controls (cf. ref. 7) (Fig. 1). Five of the six merozoite immunised animals were subsequently challenged on eight occasions during a period of up to 10 months with four distinct variants and a different laboratory strain of *P. knowlesi* (Table 2). Seven of these challenges did not produce a detectable infection and viable parasites could not be demonstrated by subinoculation from Mz 10 (Table 2) after challenge with three different variants during a period of about 2 months. This animal finally developed a patent infection lasting 9 d with maximum parasitaemia of 0.02% when challenged with the W1 variant 33 weeks after initial vaccination (Table 2).

In contrast to what is observed after immunisation with parasitised cells⁵⁻⁹ or repeated infection initially controlled with drug therapy^{15,16} these results show that challenge after vaccination with merozoites derived from a single serological variant of *P. knowlesi* leads to frequent absence of detectable parasitaemia (nine out of fourteen instances); occasional sterile immunity (shown by subinoculation in two

Table 2 Merozoite (W1 variant) vaccination of rhesus monkeys against *P. knowlesi* malaria

Monkey no.	Vaccination (W1)			Challenge (10 ⁴ parasites)*					
	FCA† (day)			FIA† (day)	Day‡	Parasite	Parasitaemia Days patent	Max per 10 ⁴ RBC	Subinoculation (day)§
Mz 12	5 × 10 ⁸ (1)	2 × 10 ⁹ (75)	1 × 10 ⁹ (82)		98	W1		Neg	
Mz 10	5 × 10 ⁹ (1)	6 × 10 ⁹ (14)	5 × 10 ⁸ (37)		74	W1		Neg	S (85)
					93	W42		Neg	
					127	W12		Neg	S (133)
					232	W1	242—251	2	1 (259)
Mz 16	6 × 10 ⁸ (1)	9 × 10 ⁸ (14)	2 × 10 ⁹ (62)	5 × 10 ⁹ (98)	280	N1		Neg	
					113	W1	131—137	4	1 (124)¶
Mz 14	6 × 10 ⁸ (1)	9 × 10 ⁸ (14)	2 × 10 ⁹ (62)		148	W3		Neg	
					76	W3	81— 89	7	
Mz 15	6 × 10 ⁸ (1)	9 × 10 ⁸ (14)	2 × 10 ⁹ (62)		118	W42		Neg	
					76	W3	83— 95	150	
Mz 17	6 × 10 ⁸ (1)	9 × 10 ⁸ (14)	2 × 10 ⁹ (62)	5 × 10 ⁹ (98)	118	W42		Neg	
					112	W3	118;131—139	28	
					148	W1		Neg	

*For details of parasites see footnote Table 1.

†No. merozoites in Freund's complete (FCA) or incomplete (FIA) adjuvant.

‡Days refer to time after first vaccination.

§Subinoculation of 5 ml blood from vaccinated into normal rhesus on day shown. S, No infection; I, infection in recipient.

||Challenge with 10^6 parasites.

¶Infection in recipient of blood was due to a *P. knowlesi* variant other than W1.

instances); parasitaemia of short duration (6–12 d) and relatively low intensity (maximum 0.02–1.5%) when patent infection did occur (five out of fourteen instances); resistance to several serological variants different from that used for immunisation. The available data suggest that the various schedules of vaccination employed (Table 2) provided comparable protection; we are now attempting to establish whether adjuvant is essential for merozoite vaccination. The number of merozoites was chosen arbitrarily; a smaller number might be effective, but at the level used, the *in vitro* cultures provide about 20 immunising doses per ml parasitised red blood cells. The induced immunity seems to be predominantly species specific since 3 merozoite immunised animals challenged with 10^5 *P. cynomolgi bastianellii* parasites developed chronic patent infections, although maximum parasitaemia was lower than in normal monkeys (Table 3). *P. knowlesi* infections controlled by drug therapy do not cross immunise against *P. cynomolgi bastianellii*¹⁷ which produces chronic, non-fatal malaria in rhesus monkeys.

We conclude that merozoite vaccination protects rhesus monkeys against initial homologous or heterologous variant infection with erythrocytic stages of the normally lethal *P. knowlesi* parasite. Vaccinated animals once challenged are also resistant to subsequent infection with other variants or a different laboratory strain of *P. knowlesi*. Merozoite vaccination induces immunity far greater in degree and significantly broader in specificity than previously achieved by immunisation or repeated infection. This form of vac-

Table 3 *P. cynomolgi bastianellii* challenge (10^5 parasites) of normal controls and monkeys vaccinated with *P. knowlesi* merozoites

Monkey	Day of challenge*	Pre-patent period (d)	Maximum parasitaemia per 10^4 RBC	Duration of patent infection (d)
389-normal		5	1200	> 80
391-normal		4	1250	> 80
Mz10(†)	323	12	68	> 80
Mz14(†)	142	4	400	> 80
Mz15(†)	142	5	550	> 80

*Day after initial vaccination on which *P. cynomolgi bastianellii* challenge was administered.

†See Table 2 for previous immunisation and challenge of merozoite vaccinated animals.

cination apparently stimulates a specific response to antigens present on a wide spectrum of *P. knowlesi* parasites, but poorly inductive, during natural malaria infection. Our observations are significant with regard to the development of an effective vaccine against human malaria in view of the probable existence of immunologically distinct strains of *P. falciparum*^{18,19} and the established genetic diversity of this parasite in West Africa²⁰.

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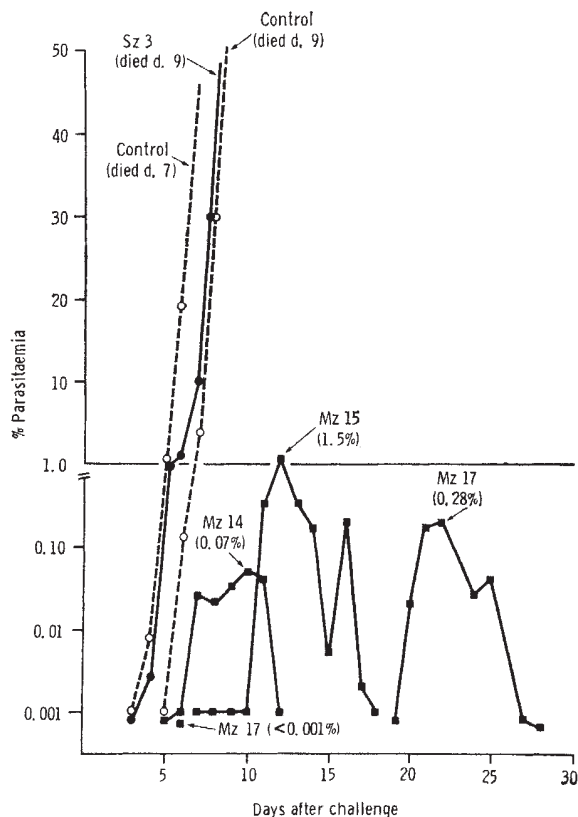


Fig. 1 Course of parasitaemia in two normal rhesus monkeys, one immunised with schizonts (Sz 3), and three immunised with merozoites (Mz 14, 15 and 17, Table 2). All animals were challenged with 10^4 *P. knowlesi* parasites (W3 variant). Sz 3 had been vaccinated with a total of 1.3×10^6 erythrocytic schizonts (W1 variant) on days 1, 41 and 77 and was challenged on day 91. To illustrate the course of low-grade infections in the merozoite immunised animals, parasitaemia (vertical axis) is shown on a logarithmic scale between 0.001 and 1.0%.

Excystment by sporozoites of malaria parasites

It is widely assumed that the mature oocyst of the malaria parasite bursts and liberates the sporozoites *en masse*¹ although studies on the intestinal coccidians have clearly shown that the sporozoites escape through the wall of the sporocyst, a structure analogous to the oocyst of malaria parasites, by way of specialised pores²⁻⁴. During a scanning electron microscope study of the sporogonic cycle of the rodent malaria parasite *Plasmodium yoelii nigeriensis* in the vector *Anopheles stephensi*, evidence has been obtained which suggests that excystment of the sporozoite may occur by a method differing from both those described above.

Examination of oocysts on a very heavily infected gut prepared 11 d after infection revealed the presence of small holes, 0.25–0.65 µm in diameter, in the wall of some cysts (Fig. 1a). Furthermore these perforations were limited to areas some 15 µm across on oocysts 40 µm in diameter. The holes were found with increasing frequency towards the centre of this region, where larger perforations appeared. Sporozoites could be seen emerging individually through these holes. While this might have represented the early stages of the total destruction of the oocyst, evidence to the contrary was provided by the occurrence of empty oocysts in which only small regions of the cyst wall were damaged (Fig. 1b). The basal lamina of the mosquito gut epithelium lay as a diaphanous netting over the surface of most oocysts, but frequently could not be detected at all. Should this reflect the *in vivo* condition the basal lamina can be considered little obstacle to the escaping sporozoites.

These observations suggest that sporozoites escape through the oocyst wall by small perforations which become enlarged as emergence progresses (Fig. 1a and b). In some instances this process may be traumatic enough to destroy the oocyst. Further, it is clear there is no organelle in the wall of malaria parasite oocysts through which the sporozoites emerge, comparable to the pore occupied by the Stieda body in the sporocyst wall of intestinal coccidians.

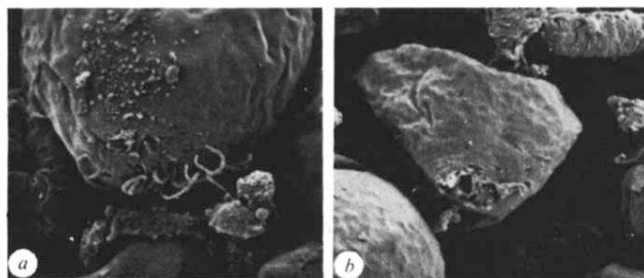


Fig. 1 a, Scanning micrograph showing sporozoites escaping from perforated oocyst of *Plasmodium yoelii nigeriensis* on the midgut of *Anopheles stephensi*. The infection was maintained for 11 d at 25°C and 80% relative humidity. The specimen was dissected into Schneider's *Drosophila* medium and rinsed briefly. Following fixation in 3% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 the specimen was washed in 0.15 M phosphate buffer and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer. Dehydration was in acetone. Samples were dried in carbon dioxide (using the critical point method) and gold coated in a 'sputter coater' ($\times 60$). b, Micrograph of oocyst following the escape of the sporozoites. ($\times 12$).

While it is recognised that the preparations examined bore unusually high densities of oocysts, and the consequent pattern of sporozoite release need not be identical to that normally seen, it is still necessary to explain both the direction and the restricted path of migration of the sporozoites through the oocyst wall. The breakdown of the oocyst could be a passive process determined by areas of weakness in the cyst wall. Alternatively the sporozoites may penetrate the oocyst actively, their orientation to areas of cyst wall that lie adjacent to the haemocoel being determined by some biochemical gradient between the surrounding haemocoelomic fluid and midgut epithelium of the

mosquito. In the present study the intimate contact of the adjacent oocysts could severely limit these areas of the cyst wall in contact with the haemocoelomic fluid.

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Evidence for energy-dependent accumulation of paraquat into rat lung

THE herbicide paraquat (N,N'-dimethyl 4,4'-bipyridilium) can produce widespread oedema and fibrosis in the human lung after accidental ingestion¹⁻³. In those cases where death occurs after several weeks, there are no apparent pulmonary changes during the first few days following ingestion. Animal experiments in a variety of species have shown the lung to be the major target organ⁴⁻⁶. After administration of paraquat to animals, the lung has a high initial concentration and retains paraquat⁷⁻⁹. This retention appears to be related to the development of lung damage⁷ (L. L. Smith and M. S. Rose, unpublished work). The mechanism of retention of paraquat by the lung is at present not understood.

We have demonstrated an energy-dependent accumulation of paraquat in slices of rat lung. This process may account for the retention of paraquat in the lungs of many species. Diquat (N,N'-ethylene 2,2'-bipyridilium), a herbicide closely related in structure and properties to paraquat, is not actively accumulated by lung slices, is not retained by the lung *in vivo*^{7,8} and does not damage the lung¹⁰ (L. L. Smith and M. S. Rose, unpublished work).

Male Alderley Park (Wistar derived) specific pathogen free rats (body weights 170–200 g) were killed with halothane. The lungs were removed and slices prepared (20–70 mg wet weight) at room temperature. Only slices with two cut surfaces were used. Incubation was carried out at 37°C in 3.0 ml Krebs Ringer phosphate¹¹ containing 200 mg% (w/v) glucose, 0.1 µCi of methyl-¹⁴C-paraquat or 0.1 µCi of ethylene-¹⁴C-diquat (Radiochemical Centre, Amersham; specific activity for both approximately 30 mCi mmol⁻¹), and the required concentration of non-radioactive paraquat or diquat dichloride. After incubation, slices were washed by transfer to fresh medium without bipyridyl, blotted and dissolved in 1.0 ml Soluene (Packard Instrument Company Limited). Scintillator [10 ml Dimilume (Packard Instrument Company Limited)] was added and the radioactivity of the slice measured using a liquid scintillation spectrometer. 0.1 ml of media was diluted to 1.0 ml with water and the radioactivity measured after addition of 10 ml Instagel scintillator (Packard Instrument Company Limited).

The amount of diquat found in rat lung slices incubated in media containing diquat (10^{-5} – 10^{-3} M) remained constant from 30 min to 2 h (Fig. 1a). The plateau value obtained was dependent on the concentration present in the medium (Fig. 1a). In contrast, paraquat was accumulated linearly from 30 min to 2 h (and in experiments not reported here, for up to 4 h) (Fig. 1b). The amounts of paraquat accumulated were in excess of those seen with diquat. When KCN (10^{-3} M) plus iodoacetate (10^{-3} M) were added to the medium either at the beginning of the incubation or after 1 h, this continued

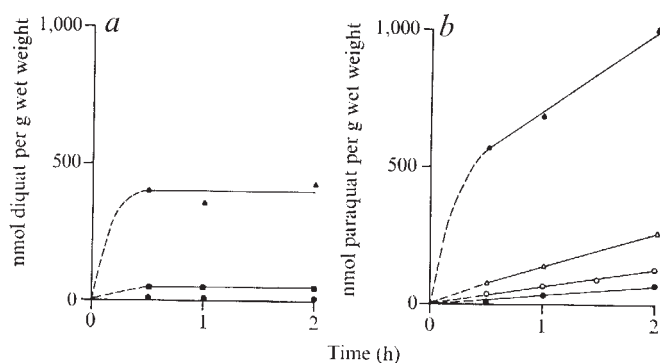


Fig. 1 The accumulation of paraquat and diquat by slices of rat lung. The medium contained the following concentration of bipyridyl: ●, 1×10^{-5} M; ○, 2×10^{-5} M; △, 4×10^{-5} M; ■, 1×10^{-4} M; ▲, 1×10^{-3} M. Individual points are the mean values from four slices.

accumulation of paraquat was inhibited (Fig. 2a). Identical inhibition has been demonstrated with both 10^{-5} and 10^{-4} M paraquat present in the medium. Rotenone (10^{-5} M) a specific inhibitor of mitochondrial respiration also inhibits uptake of paraquat (Fig. 2b). Thus, the accumulation of paraquat into slices of rat lung to amounts in excess of those seen with diquat is energy-dependent. A plot of rate of uptake (v) against the rate divided by the concentration of paraquat in the medium ($\frac{v}{c}$) is linear (Fig. 3). From these data, an apparent

K_m of 7×10^{-5} M and a V_{max} of 300 nmol paraquat per g wet weight per h can be calculated for this process.

After oral administration of 126 mg paraquat per kg body weight to rats, the lung concentration was shown to rise from approximately 4 μ g per g wet weight at 4 h to approximately 14 μ g per g wet weight at 32 h (ref. 9). During this time, the plasma concentration was constant at around 1 μ g ml $^{-1}$. Paraquat is removed rapidly from the blood of rats after intravenous administration⁷. The maintenance of a constant concentration of 1 μ g ml $^{-1}$ for approximately 30 h can, therefore, only be the result of release of paraquat from other organs into the blood, or impaired renal function, or both. Since the gastrointestinal tract of rats was shown to contain a large proportion of the oral dose⁹, this is the most likely source of the blood paraquat. The lung was clearly able to accumulate paraquat to levels in excess of the blood concentration. Similar experiments with diquat given orally to rats show that no such accumulation occurs (L. L. Smith and M. S. Rose, unpublished

work). Thus the rat lung accumulates paraquat but not diquat both *in vivo* and *in vitro*.

Since it is known that the response of human lung to oral paraquat is delayed, it is possible that a similar accumulation process occurs in man. Therefore, it is of paramount importance that, after ingestion of paraquat, all possible measures are taken to remove paraquat not only from the stomach, but also from the rest of the gastrointestinal tract and blood.

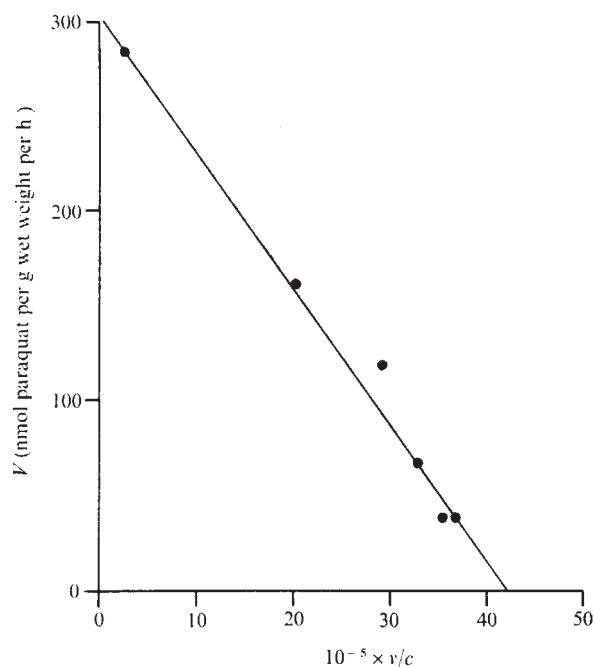


Fig. 3 The derivation of an apparent K_m and V_{max} for the uptake process.

Further work is in progress in these laboratories to investigate the mechanism of this uptake of paraquat into the lung. If the process can be inhibited *in vivo* it may be possible to prevent lung damage.

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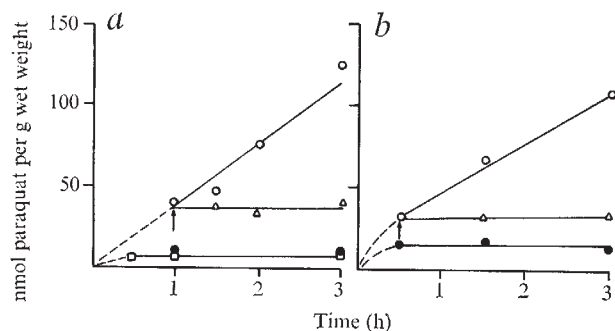


Fig. 2 The effect of metabolic inhibitors on the accumulation of paraquat by slices of rat lung. *a*, The medium contained: ○, paraquat (1×10^{-5} M); □, diquat (1×10^{-5} M); ●, paraquat (1×10^{-5} M) plus KCN (10^{-3} M) plus iodoacetate (10^{-3} M); △, paraquat (1×10^{-5} M) plus KCN (10^{-3} M) plus iodoacetate (10^{-3} M) added at arrow. *b*, The medium contained: ○, paraquat (1×10^{-5} M); ●, paraquat (1×10^{-5} M) plus rotenone (1×10^{-5} M); △, paraquat (1×10^{-5} M) plus rotenone (1×10^{-5} M) added at the arrow. Individual points are the mean values from two slices.

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Phytoalexin production by live cells in broad bean leaves infected with *Botrytis cinerea*

STUDIES on the role of phytoalexins in disease resistance in plants have been limited by the absence of techniques for the accurate localisation of the inhibitors within infected tissues. As a result the postulate of Müller and Börger¹ that phytoalexin production is confined to cells undergoing necrobiosis remains in doubt².

The closely related phytoalexins wyerone and wyerone acid accumulate in leaves of broad bean following fungal infection^{3,4}. Although Deverall and Vessey⁵ suggested that phytoalexins from *Vicia faba* might be produced by healthy cells, further work by Mansfield and Deverall⁶ led them to argue that wyerone acid production was confined to cells undergoing necrobiosis and browning. Here we report the use of fluorescence microspectrography to confirm that wyerone acid is produced by living cells in bean leaves infected with *Botrytis cinerea*.

Leaves were detached from field grown plants of broad bean cv. the Sutton and inoculated on the lower epidermis with 10 µl droplets of sterile distilled water each containing about 5,000 conidia of *B. cinerea*, or water alone⁷. Sites inoculated with *B. cinerea* were flecked dark brown after 2 d when droplets were collected and the underlying epidermis peeled off and stored at -20° C. Both wyerone and wyerone acid accumulated in infected epidermal tissues but only the acid was found in inoculum droplets (Table 1). Assuming that wyerone acid in droplets originated in the epidermis the ratio of wyerone acid:wyerone produced by epidermal cells was about 21:1. The phytoalexins could not be detected in leaves inoculated with water alone.

Examination of epidermal strips by fluorescence microscopy revealed that cells that had undergone browning in response to infection by *B. cinerea* absorbed ultraviolet light but many adjacent healthy cells fluoresced blue/green. The vitality of the fluorescing cells was confirmed by their plasmolysis in 2 M sucrose and accumulation of neutral red vital stain. Fluorescence was associated with the cell vacuole. No fluorescent cells were observed in epidermis inoculated with water alone.

Both wyerone and wyerone acid fluoresce under ultraviolet light and these compounds were identified as the possible cause of the fluorescence in infected tissues by microspectrography. Examples of the almost identical spectra obtained from fluorescent cells and solutions of the purified phytoalexins are given in Fig. 1. Similar spectra were obtained for ethanolic solutions of both wyerone and wyerone acid on a standard spectrophotofluorometer (Aminco-Bowman 4-8202SPF). In view of the much greater concentrations of wyerone acid than wyerone

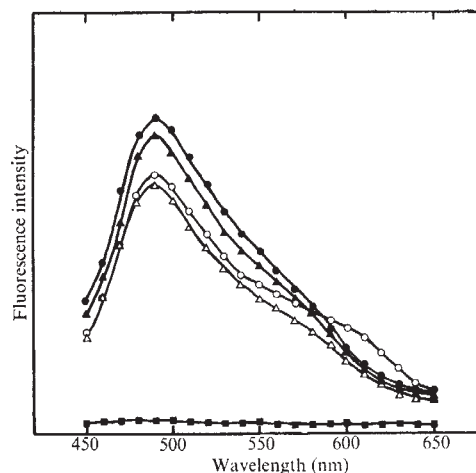


Fig. 1 Fluorescence emission spectra recorded with a Leitz microspectrograph using xenon lamp 405 nm excitation. Spectra recorded for the vacuolar region of different fluorescent cells before (●) and after (○) plasmolysis in epidermal tissue inoculated with *B. cinerea* and a cell from uninfected epidermis (■). Spectra of wyerone (Δ) and wyerone acid (▲) dried on to microscope slides from solution in ethanol and mounted in distilled water.

extracted from epidermal tissues it is probable that the fluorescence of live cells was largely caused by wyerone acid in the cell vacuole.

The presence of wyerone acid in live cells could result from its production by these cells or accumulation from adjacent necrotic cells and surrounding intercellular fluids. The occurrence of isolated, brightly fluorescent cells, however, rather than a zone of fluorescent tissue around necrotic sites strongly suggested that individual cells were actively synthesising the phytoalexin. The fluorescence of wyerone acid in necrotic cells may have been masked by the accumulation of ultraviolet-absorbing substances during cell browning. We conclude that wyerone acid can be produced by live cells within infected tissue.

These results raise several possibilities concerning the relationship between host cell death and the production of wyerone acid by the broad bean plant. Phytoalexin production may be triggered initially by cell death in response to fungal invasion and metabolites released from dead cells may induce subsequent biosynthesis of wyerone acid in adjacent living cells. Alternatively fungal metabolites may act as specific inducers of phytoalexin production which may be confined to live cells where wyerone acid may ultimately reach phytotoxic concentrations and cause host cell death. It is hoped that further use of the microspectrograph and detailed microscopical observations on the early stages of infection will allow these possibilities to be examined.

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Table 1 Yield of wyerone and wyerone acid from inoculum droplets and underlying epidermis collected from 200 inoculation sites

	Yield (µg)		Yield per g fresh epidermal tissue (µg)	
	Wyerone	Wyerone acid	Wyerone	Wyerone acid
Droplet	0	20.0	0	182
Epidermis	2.4	30.5	21.8	277
Total	2.4	50.5	21.8	459

All yields are the mean of results from two experiments. Epidermal tissue (0.1 and 0.12 g) was ground in methanol in a hand homogeniser. The methanol extract was evaporated to dryness *in vacuo* and partitioned three times between water (5 ml) and diethyl ether (10 ml). Wyerone and wyerone acid were purified from the ether soluble fraction by thin layer chromatography (TLC) on Merck silica gel precoated plates 0.25 mm thick developed in saturated tanks with diethyl ether: methanol (4:1, 8 cm run) then in dichloromethane: petroleum ether 60-80° C (2:1, 16 cm run). Wyerone and wyerone acid were detected as bands fluorescing blue under ultraviolet light (wavelength 366 nm) at final *R_f* 0.6 and *R_f* 0.3, respectively. After elution from silica gel with methanol the concentrations of the phytoalexins were recorded by ultraviolet spectrophotometry³. Inoculum droplets (1.1 and 0.9 ml) were partitioned three times with 5 ml diethyl ether and the phytoalexins isolated from the ether phase by TLC as described above.

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Data processing by the chemotaxis machinery of *Escherichia coli*

THE chemotactic behaviour of *Escherichia coli* provides a useful model for study of the molecular basis of a simple stimulus-response sequence¹. Chemical stimuli are detected by specific receptors² which in turn alter the rotation of the flagella³ to elicit movement towards attractants or away from repellents. About twenty types of chemoreceptors have been identified in *E. coli*⁴⁻⁶, suggesting that a communication system transmits sensory data from the receptors to the flagella, and that signals from different receptors converge either before or on reaching the flagella. I have attempted to dissect this system by isolating and examining nonchemotactic (*che*) mutants of the type first described in *E. coli* by Armstrong *et al.*⁷. In this report I summarise the properties of *che* mutants and derive a model of the chemotaxis machinery based on this genetic analysis.

Chemotaxis in *E. coli* is mediated through variations in the frequency of directional changes (tumbles) that occur during swimming^{8,9}. When bacteria sense an increase in attractant concentration⁸⁻¹⁰, or a decrease in repellent concentration¹¹, tumbling is suppressed, causing net migration in the desired direction. Berg and Brown⁸ proposed that tumbles are initiated by a 'tumble generator' and that tumble frequency is controlled by chemoreceptor signals that modulate this generator. Since *E. coli* swim by rotating their flagellar filaments^{12,13}, tumbling depends on the direction of rotation³. Counter-clockwise rotation causes straight swimming; reversal to a clockwise direction initiates tumbling.

Chemotaxis mutants that are motile but cannot respond to any stimuli (*che* mutants) should define common components of the chemotaxis machinery, including the tumble generator, provided that such components are not an essential part of the flagellum or its motor. Table 1 summarises the swimming behaviour of the *che* mutants studied by Armstrong *et al.*^{7,14} and myself (in preparation).

All the mutants, which represent four genes, exhibit aberrant swimming patterns due to incessant tumbling or loss of tumbling. This result indicates that most or all of the common components of the chemotaxis machinery that are not essential for viability are required for generating and regulating tumbles.

Table 1 Properties of generally nonchemotactic (*che*) mutants

Complementation Class*	No. of independent isolates	Swimming pattern†
<i>cheA</i>	16	No tumbling
<i>cheB</i> (type 1)	21	No tumbling
<i>cheB</i> (type 2)	—	Incessant tumbling
<i>cheC</i>	1	No tumbling
<i>cheD</i>	—	No tumbling

*In the original complementation study of Armstrong and Adler¹⁴, gene assignments were made on the basis of abortive transduction tests. These assignments were confirmed† using F-prime episomes to construct stable partial diploids.

†This work is in preparation.

‡Bacteria were grown at 35° C in tryptone broth to an absorbance at 590 nm of 0.3, and were either observed directly with the light microscope or were first washed and resuspended in 10 mM potassium phosphate (pH 7) plus 0.1 mM EDTA. Swimming patterns are the same in either case.

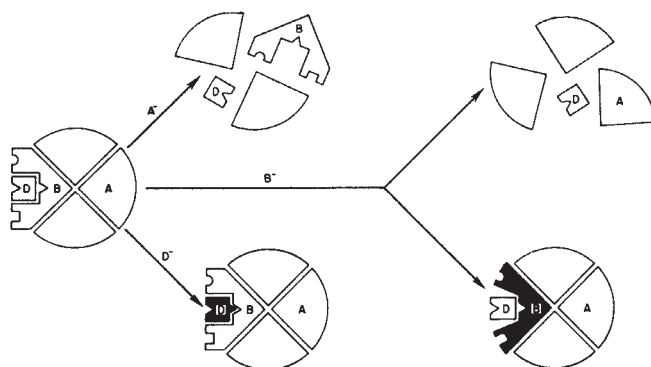


Fig. 1 A model of the tumble generator based on the properties of generally non-chemotactic (*che*) mutants. A further explanation is given in the text.

Although mutations in the *che* genes can lead to altered tumbling patterns, this does not necessarily mean that the product of each gene is required directly for tumbling. *Che* mutants might define not only parts of the tumble generator but also components that interact with the generator to modify tumbling behaviour. The role of each *che* gene product can best be inferred from mutants that lack entirely the product or function of a *che* gene. As the null phenotypes of the *che* genes reveal (Table 2), several *che* gene products are not involved directly in generating tumbles. The product of the *cheC* gene seems to be an essential component of the flagellum since Silverman and Simon¹⁵ showed that in its absence the flagellum is not assembled. Motile, nonchemotactic *cheC* mutants cannot tumble, and must represent special alterations of the *cheC* product that permit assembly of a flagellum that can rotate only counter-clockwise.

The product of the *cheD* gene is not required for tumbling, since in its absence, tumbling is normal although chemotaxis to serine^{5,16} and some repellents⁸ is lost. But, some alterations of the *cheD* product do lead to a loss of tumbling ability. Since these mutants are invariably dominant (my work in preparation), it seems that an appropriately altered *cheD* product can interact with and inhibit the tumble generator.

Table 2 Phenotypes of null mutants in the *che* genes

Gene	Swimming phenotype	Chemotaxis phenotype
<i>cheA</i>	No tumbling	Generally non-chemotactic
<i>cheB</i>	No tumbling	Generally non-chemotactic
<i>cheC</i>	Nonmotile	Non-chemotactic (no flagella) ¹⁵
<i>cheD</i>	Wild type ⁸	Specifically non-chemotactic to serine ^{5,16} and to some repellents ⁸

Null mutants are those with a complete loss or with total inactivity of a particular gene product. The null phenotypes of the *che* genes were determined in various ways, most of which involve the study of nonsense or deletion mutants in these genes (my work in preparation).

Thus the normal *cheD* product may function as a 'switch' to control the tumble generator in response to serine signals and some repellent signals. In nonchemotactic *cheD* mutants this switch may be frozen in the 'off' position to prevent tumbling.

The products of the *cheA* and *cheB* genes are probably part of the tumble generator since null mutations in these genes result in a nontumbling phenotype (Table 2). The *cheB* product, however, seems to have a dual role which is reflected in the two tumbling patterns of *cheB* mutants (Table 1). The non-tumbling *cheB* mutants indicate that *cheB* product is needed to generate tumbles, and the incessantly tumbling *cheB* mutants indicate that *cheB* product also plays a part in regulating tumble frequency.

What is the nature of the defect in the incessantly tumbling (uncoordinated¹⁷) *cheB* mutants? On the one hand, tumbling mutants might have hyperactive tumble generators, and if this is the case, each mutant should respond equally well or poorly

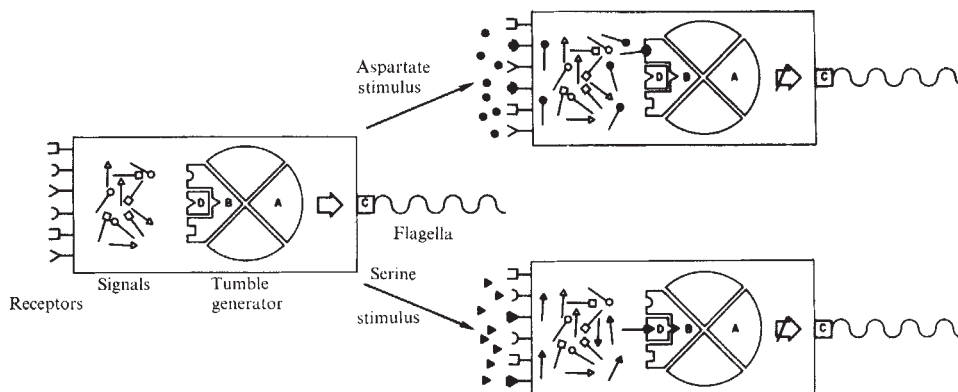


Fig. 2 The role of the tumble generator in chemotaxis. A further explanation is given in the text.

to different stimuli. On the other hand, tumbling mutants might be poorly coupled to the receptor signals that control tumbling rates, as Berg and Brown suggested⁸. In this case, the tumbling frequency of the mutants represents not an over-active generator but rather a free running generator, unmodulated by receptor signals. If the tumble generator in these mutants is defective in its response to modulating signals, different signals might be perceived with different efficiencies and thereby elicit different responses.

Tumbling mutants respond to sudden changes in attractant concentration although not as well as wild type (Table 3 and refs 3 and 18). In most cases tumbling mutants do not respond with the same efficiency to the attractants aspartate and serine which are detected by different chemoreceptors. Some mutants (e13p1, e16f1, e19e1, e27f1) respond to serine but respond poorly or not at all to aspartate; other mutants (e16h1, e16n2, e17a1, e20t2, e26e2, e28h2) respond to both types of stimuli,

and one (e21e1) responds poorly to both. Comparisons between mutants (for example, e17a1 with e27f1 or e16n2 with e28h2) show that two mutants can have the same response to one stimulus but a different response to another stimulus. These results suggest that tumbling mutants cannot be modulated properly by chemoreceptor signals and that these signals control tumbling frequency by interacting with the *cheB* product.

A model of the tumble generator based on these findings is presented in Fig. 1. The generator has three components (and perhaps others not yet detected by mutation) each of which can mutate to produce a generally nonchemotactic phenotype in one or more ways. The *cheC* product is assumed to be a component of the flagellum rather than a part of the tumble generator itself. The products of the *cheA* and *cheB* genes are essential components of the tumble generator. Both are required to generate tumbles, but the *cheB* product also controls tumbling rate by interacting with signals from the chemoreceptors. The *cheD* product is also thought to be a component of the tumble generator although the *cheD* product is not required for tumbling. Rather, the *cheD* product is a component of the tumble control system, passing serine signals and some repellent signals to the tumble generator.

Since the *cheB* product is ultimately responsible for controlling tumbling rate in response to chemoreceptor signals, the model assumes that the *cheD* product interacts directly with the *cheB* component in transmitting signals to the tumble generator. This interaction can be demonstrated in three ways. First, it is possible to isolate suppressors of *cheD* mutants that are mutations in the *cheB* gene. Second, double mutants containing a *cheB* tumbling mutation and a *cheD* nontumbling mutation tumble either incessantly or not at all depending on the severity of the *cheB* defect as measured by temporal stimulation. Third, the response of tumbling *cheB* mutants to aspartate stimuli can be altered greatly by *cheD* null mutations, suggesting that the configuration of the *cheB* product can be changed by removing the *cheD* product from the generator. (A complete description of experiments on the interaction of the *cheB* and *cheD* products will be presented separately.)

The postulated role of the tumble generator in chemotaxis is shown in Fig. 2. The tumble generator is probably controlled in a negative manner by inhibitory signals whose level is modulated by the various chemoreceptors. In the absence of stimuli, the level of inhibitory signals is low, and consequently tumble impulses are frequently sent to the flagella. The level of inhibitory signal rises whenever stimuli are detected by the chemoreceptors; different receptors control different inhibitory signals. Aspartate signals (and presumably several others) interact directly with the *cheB* component, whereas serine signals and some repellent signals are transmitted to the tumble generator through the *cheD* product.

When *E. coli* are presented simultaneously with an attractant and a repellent the response depends on the relative strengths of the conflicting stimuli^{11,19}. The model of the chemotactic process presented in Fig. 2 implies that opposing stimuli can be integrated at several points depending on the nature of the

Table 3 Swimming behaviour of tumbling mutants in response to temporal stimulation with the attractants aspartate or serine

Tumbling mutant†	Duration (s) of response to attractant jumps*			
	Aspartate		Serine	
	3-fold jump	30-fold jump	3-fold jump	30-fold jump
Wild type	101 ± 12 (7)	122 ± 10 (3)	346 ± 38 (4)	> 600 (3)
e13p1	None (3)	None (5)	98 ± 7 (3)	100 ± 13 (5)
e16f1	None (3)	None (5)	65 ± 10 (5)	89 ± 6 (3)
e16h1	62 ± 8 (5)	88 ± 5 (2)	121 ± 16 (5)	188 ± 10 (2)
e16n2	79 ± 13 (5)	112 ± 7 (2)	167 ± 28 (5)	243 ± 12 (2)
e17a1	84 ± 9 (5)	111 ± 8 (2)	94 ± 7 (5)	144 ± 11 (2)
e19e1	None (3)	50 ± 7 (5)	64 ± 12 (5)	107 ± 11 (3)
e20t2	67 ± 12 (5)	109 ± 13 (2)	140 ± 12 (5)	187 ± 11 (2)
e21e1	None (3)	None (3)	None (3)	66 ± 11 (5)
e26e2	43 ± 5 (5)	96 ± 18 (3)	74 ± 9 (5)	107 ± 4 (2)
e27f1	None (5)	61 ± 4 (2)	99 ± 17 (5)	154 ± 13 (2)
e28h2	63 ± 7 (5)	99 ± 10 (2)	111 ± 12 (5)	183 (1)

*Bacteria were grown at 35°C in minimal salts-glycerol medium to an optical density at 590 nm of 0.2. The cells were washed and resuspended in 10 mM potassium phosphate (pH7) containing 0.1 mM EDTA at an optical density at 590 nm of 0.1. Samples of the bacteria were then incubated at room temperature for 15 min in the presence of 0.1 mM attractant (either aspartate or serine) before an experiment began. To minimise changes in background attractant concentrations, each set of measurements was completed within 15 min thereafter. Drops containing 20 µl of bacteria in attractant were placed on microscope slides and incubated for 1 min. The cells were then stimulated by the addition of 1 µl of attractant, bringing the final concentration to 0.3 mM (3-fold jump) or to 3 mM (30-fold jump). If the bacteria could respond, they stopped tumbling and began to swim in straight lines for a period that varied directly with the stimulus size. The times shown are subjective estimates of the time it took for 50% of the responding cells to begin tumbling once again. Differences between independent observers were not significant and the data were pooled to give the averages and standard deviations and (in parentheses) the number of measurements made. These estimates are quite accurate for tumbling mutants because the two behaviours—swimming and tumbling—are very distinct and the transition time is short relative to the response time. The wild type is more difficult to measure because its normal tumble frequency is not as great as in tumbling mutants.

†Each of these tumbling mutations is in the *cheB* gene. All the mutant alleles were transduced into a common genetic background for these experiments. Mutants e20t2, e26e2, and e27f1 are *amber* (nonsense) mutants.

stimuli. For example, the chemoreceptors for serine and for some repellents may control the same signal, in which case integration would take place at the signal level. If different signals are controlled by these receptors, they would be integrated at the *cheD* product. Other combinations of stimuli, for example, aspartate and a repellent, would be integrated by the *cheB* product.

These studies of chemotaxis mutants indicate that at least two types of chemoreceptor signal are transmitted to the tumble generator. Although nothing is known about these signals, it seems unlikely that they will prove to be electrical unless each signal is associated with a different class of ions. Perhaps chemoreceptors communicate with the tumble generator by mechanical or chemical means. In any event, the central role of these signals in the chemotactic process emphasizes the importance of learning more about their nature and their mode of transmission.

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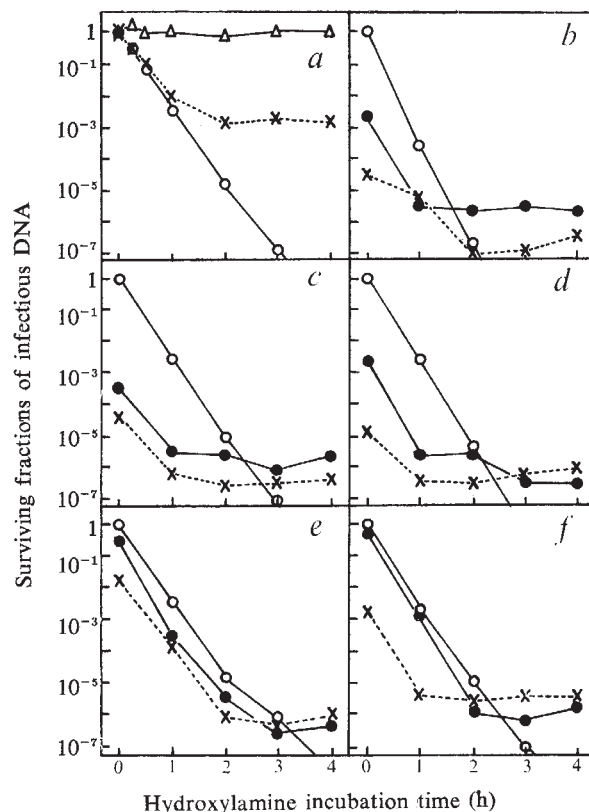


Fig. 1 Hydroxylamine treatment of the infectious materials extracted from Φ X-SS-DNA-transfected spheroplasts. The bacterial spheroplasts (10^9 cells ml^{-1}) were incubated with SS-DNA (10^{12} molecules ml^{-1}) for 15 min and then PAM medium was added and incubation was continued further (see legend of Table 1). At intervals, spheroplasts were lysed by the addition of 1/20 volume of 12.5% Sarkosyl NL97 and the lysates were extracted by phenol and nucleic acids were precipitated by alcohol, resuspended in 0.05 M Tris-HCl, pH 6.0, added with 1/5 volume of 1 M hydroxylamine-HCl, pH 6.0, and incubated at 37° C. At intervals, aliquots were diluted and their surviving infectivity was assayed on *E. coli* K12W6 spheroplast. The surviving fraction was expressed as the ratio of PFU in spheroplast assay at the indicated time of hydroxylamine treatment to PFU of the sample not treated with hydroxylamine in which SS-DNA was added to spheroplasts previously lysed with Sarkosyl and phenol. ●, 15 min incubation before the addition of PAM medium; ×, 15 min incubation after the addition of PAM medium; ○, SS-DNA added to the previously lysed spheroplasts. a, *E. coli* K12W6, Purified SS-DNA (○), RF-DNA (△) and SS-, RF-DNA mixture (×), incubated with 0.2 M hydroxylamine-HCl, pH 6.0, at 37° C. b, *K. pneumoniae*; c, *S. marcescens*; d, *P. vulgaris*; e, *P. aeruginosa*; f, *E. coli* K12W6.

Transfection of non-host bacterial spheroplasts with bacteriophage Φ X174 DNA

BACTERIOPHAGE Φ X174 containing circular single-stranded DNA (SS-DNA) can infect *Escherichia coli* C and *Shigella*¹⁻⁴. Progeny phages are produced through synthesis of circular double-stranded DNA (replicative form, RF-DNA) as the intermediate^{5,6}. Φ X174 cannot infect bacteria which have no specific receptors for phage adsorption on the cell wall. The host range in Φ X infection is thought to be determined primarily by success or failure of adsorption and if most of the cell wall is removed from the bacterium, the host range is extended and Φ X-DNA can infect *E. coli* strains other than the host, and produce progeny phages. But if the recipient bacterial spheroplasts are of species distantly related to the host,

Φ X-DNA may not be able to penetrate into the spheroplasts and replicate and produce progeny phages as well as in the host spheroplast. It is known that Φ X phage replication in *E. coli* depends largely on host functions; for example, the Φ X-DNA replication is greatly affected by several genes responsible for host DNA synthesis^{7,8}. On this basis, Φ X-DNA replication in bacteria distantly related to the natural host might on the one hand fail because of incompatibilities between Φ X-DNA and bacterial gene functions or on the other hand be successful because common gene functions are widespread throughout a wide range of bacterial species. We tried to transfect Φ X-DNA to various bacteria belonging to the *Enterobacteriaceae* and *Pseudomonas aeruginosa* to examine the capability for phage production in bacteria other than the host, and found that Φ X-RF-DNA synthesis proceeded as efficiently as in the natural host in distantly related species of bacteria, but that progeny phage production was greatly reduced. Bacterial relationships determined on the basis of DNA homology.

Preparation of spheroplasts and transfection were performed

Table 1 Efficiency of transfection in various bacterial spheroplasts

Bacterium	Efficiency		DNA homology ¹⁰
	SS-DNA	RF-DNA	
<i>Escherichia coli</i> K12W6	4.1×10^{-3}	8.0×10^{-5}	101 %
B	5.3×10^{-4}	2.4×10^{-6}	100
C ⁺ Φ	2.1×10^{-4}	3.0×10^{-6}	—
<i>Salmonella typhimurium</i>	2.1×10^{-6}	2.9×10^{-8}	71
<i>Aerobacter aerogenes</i>	1.4×10^{-8}	3.5×10^{-9}	51
<i>Klebsiella pneumoniae</i>	$4.5 \times 10^{-8*}$	$1 \times 10^{-10} >$	25
<i>Proteus vulgaris</i>	$1.0 \times 10^{-9*}$	$1 \times 10^{-10} >$	14
<i>Serratia marcescens</i>	$2.1 \times 10^{-8*}$	$1 \times 10^{-10} >$	7
<i>Pseudomonas aeruginosa</i>	$1.5 \times 10^{-9*}$	$1 \times 10^{-10} >$	1

Spheroplasts of all bacterial strains were prepared essentially according to the method of Guthrie and Sinsheimer⁹. ΦXam3-SS-DNA (10^{10} molecules ml⁻¹) in 0.05 M Tris-HCl, pH 8.1, was added to an equal volume of spheroplast stock solution in PA medium (10^8 spheroplasts ml⁻¹) and incubated for 15 min at 37° C. After an addition of eight volumes of PAM medium, incubation was continued for 120 min and then the spheroplasts were lysed with chloroform and the titre of the lysates was determined. Transfection of ΦXam3-RF-DNA (10^{10} molecules ml⁻¹) was performed in the same manner as SS-DNA. The efficiency of transfection which is the average of eight experiments is expressed as plaque forming units (PFU) obtained per input ΦX-DNA molecule. Data of DNA homology are cited from McCarthy and Bolton¹⁰.

*Values indicate averages of several experiments where plaque formation was observed.

essentially by the method of Guthrie and Sinsheimer⁹. The percentage of bacteria surviving after the osmotic burst treatment was less than 0.5% in all spheroplast preparations. In the transfection of *E. coli* K12, B and C⁺Φ (a ΦX-resistant mutant of *E. coli* C) the number of progeny phages produced was proportional to the amount of input ΦX-DNA until saturation occurred at about 10^8 ΦX-SS-DNA molecules per spheroplast. The efficiencies of transfection of ΦX-SS-DNA and RF-DNA are shown in Table 1, together with the homology of the bacterial DNA reported by McCarthy and Bolton¹⁰.

In the case of SS-DNA transfection with *Proteus vulgaris* and *P. aeruginosa* spheroplasts, phage production was sometimes not observed, probably as a result of inefficient and variable competence in transfection. The efficiency of RF-DNA transfection was lower than that of SS-DNA transfection in *E. coli* K12W6 (ref. 11) and about 50 times lower than our own results with SS-DNA. For instance, progeny phages could not be found in RF-DNA transfection in *Klebsiella pneumoniae*, *P. vulgaris*, *Serratia marcescens* and *P. aeruginosa*, probably as a result of phage production at a level too low to be detected.

We therefore examined whether ΦX-RF-DNA synthesis occurred in spheroplasts of these bacteria. To determine RF-DNA in the presence of excess input SS-DNA, DNA was extracted by phenol from spheroplasts infected with SS-DNA at intervals and treated with hydroxylamine which inactivates SS-DNA but not RF-DNA¹². The amount of RF-DNA synthesised was determined from the surviving, hydroxylamine-resistant infectivities in a spheroplast assay on *E. coli* K12W6.

Table 2 Efficiencies of RF synthesis and phage production

Bacterium	Efficiency	
	RF equivalent per spheroplast	Phage per RF equivalent
<i>E. coli</i> K12W6	2.3×10^{-2}	2.0×10
<i>K. pneumoniae</i>	2.3×10^{-3}	1.3×10^{-5}
<i>S. marcescens</i>	7.7×10^{-3}	3.2×10^{-6}
<i>P. vulgaris</i>	1.0×10^{-2}	2.4×10^{-7}
<i>P. aeruginosa</i>	2.3×10^{-2}	4.3×10^{-7}

Mean value of PFU in the spheroplast assay 3 and 4 h after the addition of hydroxylamine in Fig. 1b-f was taken as infectivity of RF-DNA. The number of RF molecules was calculated from the standard curve (not shown) of phage-yield against RF-DNA molecule in the spheroplast assay.

As shown in Fig. 1a, the infectivity of SS-DNA decreased to less than 10^{-7} after hydroxylamine treatment for 3 h, whereas that of RF-DNA was not affected. In all bacteria tested it was found that the infectivity surviving after hydroxylamine treatment for 4 h was almost as much as that of *E. coli* K12W6 (Fig. 1b-f). Decreased infectivity of input SS-DNA (see time zero of hydroxylamine treatment) was found in most bacteria tested, especially in *P. vulgaris*, *S. marcescens* and *K. pneumoniae* and is probably the result of the action of extracellular and intracellular DNases. Infectivity decreased as the time of incubation of DNA with spheroplast was increased. The frequency of hydroxylamine resistant infectious materials together with that of phage production is shown in Table 2. It seems that synthesis of RF-DNA (at least parental RF) proceeded to a similar degree in all bacteria, irrespective of phage production. Therefore, variable capability to produce progeny phage seems to be caused by a difference in blocking of later processes.

There seems to be some correlation between the efficiency of phage production and the order of bacterial DNA homology. This is not a simple reflection of difficulty of DNA penetration into the bacterial cell or degradation of ΦX-DNA by DNases, if any, before and/or after penetration, since apparently there were no great differences in the extent of RF-DNA synthesis among all the bacteria tested. It has been reported that T4 phage treated with urea transfects spheroplasts of *Salmonella anatum*, *P. vulgaris*, *S. marcescens* and *Aerobacter aerogenes* with a higher efficiency of progeny phage production¹³ than ΦX-DNA. The difference of efficiency between T4 and ΦX might reflect differences in dependence of phage growth on host functions. Phage T4 has a complex genetic composition which makes it highly autonomous compared with ΦX which is less complex and needs several host gene functions even for the conversion of SS-DNA to parental RF^{14,15}. There seem to be common factors in the machinery of RF synthesis in the bacteria tested, although the more distant is the relationship with the host, the less affinity or compatibility is there between the host functions and phage products with a concomitant reduction in phage production. The characterisation of hydroxylamine-resistant infectious material is now in progress, as is the examination of restriction as one of the possible mechanisms for a low efficiency of phage production in some bacteria.

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Selective blockage of bacteriophage T₄ assembly by chemical modification

A QUESTION of fundamental importance to structural biology is the molecular nature of the contact points which direct the protein assembly process in an extraordinarily selective and efficient manner. This problem is closely parallel to other areas of biology involving macromolecular recognition, such as enzyme/substrate specificity and antigen-antibody interaction. A promising system for the study of such structural problems is the bacteriophage T₄ which is a macromolecular

in the process of uniting the half fibres and attaching the fibres to the otherwise complete phage particle. The basic strategy employed was to subject a defective lysate to a specific modification reaction and then see if the lysate loses the ability to donate the specified phage component, in a suitable *in vitro* complementation test, while the background, completely assembled particles, survive unscathed. In this way attention was focused on critical sites exposed only in the unassembled state. To further focus on those sites which may be involved in the attachment process (as opposed to, for example, interfering with the infection process), a post-activation procedure was instituted whereby it could be ascertained not only that a modified site had blocked assembly but, furthermore, that the modified component had not been assembled to form a non-infectious particle. While hoping to study the actual contact sites, we recognise that an inherent weakness of this kind of approach is the *a priori* inability to distinguish modification at an 'active centre' from modification at a distant site which effects the observed blockage through some sort of unspecifiable conformational mechanism.

Table 1 Survey of modification reagents for selective impairment of *in vitro* complementation between fibre defective lysates

Treatment	Reference	pH	Expected target	Result
Maleic anhydride	9	7.0	Amino	Complementation reflected sensitivity of whole phage
1-fluoro-2,4-dinitrobenzene (FDNB)	10	9.0	Sulphydryl and amino	Complementation reflected sensitivity of whole phage
N-bromosuccinimide (NBS)	11	7.0	Sulphydryl and aromatic	Complementation reflected sensitivity of whole phage
N-ethylmaleimide (NEM)	9	7.0	Sulphydryl and amino	Complementation reflected sensitivity of whole phage
Woodward's reagent K	12	7.0	Carboxyl	Complementation reflected sensitivity of whole phage
Rose Bengal photooxidation	13	7.8	Imidazole	Whole phage and complementation insensitive
Iodoacetate, iodoacetamide	14	5.0	Thioether	Whole phage and complementation insensitive
Iodoacetate, iodoacetamide, iodoacetamide	14	7.0	Sulphydryl, imidazole, and thioether	Complementation impaired while control phage insensitive

For each reagent tested, defective lysates lacking proximal half fibres (34-:34am A 455), distal half fibres (37-:37am N91) or whole fibreless particles (23-:10-:23am H11/10am B255) were prepared as previously described¹⁵. The standard phosphate buffer used throughout this investigation was made of 7 g Na₂HPO₄, 3 g KH₂PO₄, 4 g NaCl and 1 l H₂O. After autoclaving, 10 ml of 0.1 M MgSO₄ was added. Before treatment, the lysates were transferred into the appropriate buffers by dialysis in the cold (4° C). The reaction was stopped and the reagent removed by exhaustive dialysis in the cold. The treatment of the control and experimental samples was identical except for the omission of the modification reagent. Exposure to the treatment buffers did not inactivate phage or interfere with subsequent complementation. A full description of the treatments may be found in the references cited in the Table. For each treated sample the titre of background phage was measured to determine the effect of modification upon phage viability. *In vitro* complementations were carried out by mixing 25 µl of the treated 34-, 37- or 23-/10- defective lysate respectively with 25 µl of (37- or 23-/10-), (34- or 23-/10-) and (37-) and incubated for 24 h at 30° C. The mixtures were then diluted with 25 ml of the standard buffer and assayed. Each complementation test of a treated lysate was accompanied by the parallel complementation of the control.

complex built up of discrete assemblies such as head, tail and tailfibres.

During the restrictive growth of conditional lethal mutants of this bacteriophage¹, phage development is blocked at the step which requires the product of the mutant gene and frequently recognisable structural components accumulate. With the demonstration that many of these components are normal phage precursors which can be used in *in vitro* studies of virus assembly², the way is open for direct biochemical investigation of the molecular basis of contact point specificities. From among the many described steps of phage assembly³, the junction of the half fibres and the attachment of the complete tail fibres to the base plate were chosen for closer scrutiny because the molecular composition of both fibre⁴ and base plate⁵ have been thoroughly examined, the contact points are topologically relatively restricted, and the reactions go well *in vitro*². The process of fibre attachment seems to pass through an intermediate in which fibres (as half or whole fibres) are transiently bound to head-related 'jig' sites⁶⁻⁸ before final binding to the base plate.

We have investigated the use of specific amino acid modification as a probe for identifying and labelling the sites involved

Various treatments employed in this study and their qualitative outcomes are summarised in Table 1. The results suggest that the assembled phage is sensitive to modification of amino, carboxyl and aromatic side groups while neither assembled phage nor fibre contact sites are sensitive to reagents specific for the thioether or imidazole side groups. Sulphydryl carboxymethylation, however, with the alkylating reagents iodoacetate, iodoacetamide and iodoacetamide manifest the requisite selectivity for the assembly process.

Post activation (Table 2) shows that the observed selective impairment of the *in vitro* complementation is indeed due to blockage of assembly. A series of experiments was carried out in which the experimental conditions (pH, reagent concentration, and duration of treatment) were varied. The results indicate that optimal discrimination between assembly blockage and phage survival is obtained by treating with 100 mM iodoacetate at pH 7.0 for 60 min at 25° C. That the three alkylating reagents used have identical effects, suggests that modification is not very sensitive to either the charge or size of the adduct. Neither the kinetics of modification (as measured by reduction in complementability) nor the kinetics of complementation with modified components, has been exhaustively

Table 2 The effect of carboxymethylation upon free fibre components

Treatment	Mutant	Component donated	Control (background) Titre Percent	Complemented with: Mutant Titre Percent
Unmodified	37-/23-/10-	Proximal half fibre	5.9×10^6 100	34- 2.0×10^8 100
Modified			5.2×10^6 89	34- 2.0×10^8 background titre = 7.6×10^6
Unmodified	34-/23-/10-	Distal half fibre	2.8×10^7 100	37- 2.8×10^9 5
Modified			2.1×10^7 75	37- 1.0×10^8 background titre = 6.0×10^6
Unmodified	23-/10-	Whole fibre	3.1×10^5 100	37- 1.4×10^9 100
Modified			4.3×10^5 138	37- 1.0×10^7 background titre = 2.2×10^6

Defective lysate preparation, modification and complementations were carried out as described in the legend for Table 1. As well as the previously described mutants, the following were used: (37-/23-/10- : 37am N91/23am H11/10am B255) in which the assembly of distal half fibres, heads and tails are blocked and (34-/23-/10- : 34am A455/23am H11/10am B255) in which assembly of proximal half fibres, head and tail is blocked. Titres are expressed as phage ml⁻¹. The left hand portion of the Table pertains to the lysates subjected to carboxymethylation. A description of each stock is followed by the titres of the background phage in the treated and parallel untreated aliquots. Percent is a normalised measure of the behaviour of a treated sample relative to the parallel untreated control aliquot. The right hand portion of the Table deals with the complementation of the indicated (untreated) defective lysates with the modified and corresponding unmodified lysates. The results of the complementations are shown both as titres of the diluted complementation mixes and as percent, which is the normalised measure of the behaviour of the modified samples with respect to their unmodified control aliquots. For postactivation, duplicate pairs of complementations involving the modified lysates were set up. After the normal 24 h incubation period, one of the pair was titrated as described and the results were as shown in this Table. To the other of the pair, an aliquot of the corresponding unmodified lysate was added and the mix was incubated a further 24 h before titration. In each case, this post activation yielded 100% phage, indicating that the original incubation with modified components did not irreversibly use up the complementary components in producing inactive phage particles.

examined. Accordingly, we are not in a position to conclude whether or not the modification is a single hit or a multiple hit phenomenon, nor whether the blockage is absolute or simply slows the assembly process.

By a suitable choice of defective lysates subjected to modification, and selection of appropriate defective lysates for *in vitro* complementation, several different subassemblies and their respective contact and 'jig' sites may be differentiated. Table 2 gives the results of experiments which examine the sensitivity to modification of the proximal half fibre, distal half fibre and the complete fibre before attachment to the base plate of the otherwise complete phage particles. Although these experiments were repeated several times, with qualitatively identical results, the details of only one set are reported here.

The striking observation to emerge from these data is that the proximal half fibre, in the absence of other fibre components seems to be insensitive to modification, while both the distal half fibre and the whole fibre are sensitive to such treatment. As the whole fibre and the distal half fibre do not have obvious, exposed assembly contact points in common, the area on the distal half fibre, which recognises the 'distal jig site', is implicated. To explore this possibility further, modification of the half fibres was done in the presence of fibreless particles. An unambiguous interpretation of the results is impossible due to the complexity of the appropriate treatment and complementation mixtures. They are consistent, however, with the notion

that the distal half fibre, when attached to the 'distal jig site', is afforded a degree of protection against modification.

In a complementary series of experiments, the modification of fibreless particles in the presence and absence of unattached half-fibres was examined and a representative set of results is presented in Table 3. It can be seen that the fibreless particles are fairly sensitive to inactivation except that a degree of protection is afforded by the proximal half fibre. This observation suggests an interaction between the proximal half fibre and the fibreless particle, with the particle half of the recognition site being susceptible to modification by alkylating reagents. Recent work (B. E. Terzaghi and E.T., unpublished) with some new mutants (fibre attachment defective) is most readily interpreted in terms of just such a distinct 'proximal jig site' transiently occupied by the proximal half fibre.

While recognising the intrinsic limitations of modification experiments, as well as the very limited amount of quantitative (kinetic) work done, the qualitative results reported here indicate that this may be a fruitful avenue of investigation. By virtue of a differential sensitivity to alkylation at pH 7.0, we have been able to confirm the existence of a distal half fibre site which recognises an assembly 'jig', as well as identifying a site on the fibreless particle which possibly serves as a 'jig' for the proximal half fibre. Thus, a handle has been found for further characterisation of a transient but possibly important type of assembly contact point.

Table 3 The effect of carboxymethylation upon fibreless particles in the presence and absence of fibre components

Treatment	Mutant	Component(s) donated	Control (background) Titre Percent	Complemented with: Mutant Titre Percent
Unmodified	X4E	Fibreless particle	1.5×10^7 100	23-/10- 3.1×10^8 100
Modified			1.8×10^7 120	23-/10- 5.9×10^7 background titre = 2.0×10^5
Unmodified	37-	Fibreless particle, proximal half fibre	1.1×10^6 100	23-/10- 2.1×10^9 100
Modified			8.8×10^5 80	23-/10- 1.4×10^9 background titre = 1.0×10^6
Unmodified	34-	Fibreless particle, distal half fibre	4.0×10^6 100	23-/10- 4.0×10^9 100
Modified			3.7×10^6 92	23-/10- 6.8×10^8 background titre = 1.2×10^7

Defective lysate preparation, modification and complementations were carried out as described in the legend for Table 1, and the format is identical to that of Table 2. In addition to the mutants already described, (X4E : 34am B25/34am A455/35am B252/37am N52/38am B262) was used as a source of fibreless particles, free of fibre components.

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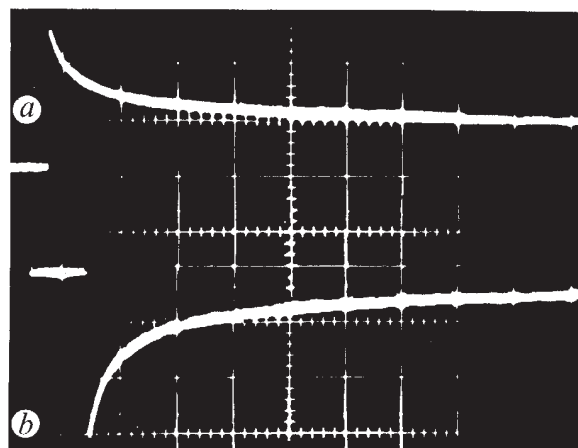


Fig. 1 Oscillograms showing changes in optical absorption and conductivity following pulse radiolysis of a nitrous oxide saturated solution of guanosine (6×10^{-4} M, pH = 4.6). Dose about 2 k rad. a, absorption at 370 nm; b, conductivity. Time scale 1,000 μ s per large division.

pH = 4-5) transient absorption spectra similar to those reported previously^{7,8} and attributable to the products of reactions of the hydroxyl radical, were observed. Except in the case of guanosine, little change in conductivity was observed over the same period. With the latter, a large decrease in conductivity occurred immediately after the radiation pulse. Such a change in conductivity in acid solution indicates the formation of positive ions and is attributed to the process:



where G = guanosine. The hydroxide ion is immediately neutralised:



with the result that a highly conducting proton ($\Lambda = 315 \Omega^{-1} \text{ cm}^2$) is replaced by a less conducting cation ($\Lambda = 50-70 \Omega^{-1} \text{ cm}^2$) with a resultant decrease in conductivity. The subsequent increase in conductivity results from the liberation of a proton with the decay of the radical-cation. The optical signal at

Interaction of dGMP radical with cysteamine and promethazine as possible model of DNA repair

THE lethal action of ionising radiation or alkylating drugs is thought to involve the formation of electrophilic species (many of which are free radical in nature) which interact with essential molecules such as nucleic acid. The protective effect of the nucleophilic compound, cysteamine, has been attributed to its ability, first, to scavenge these species thereby preventing their reaction or, second, to reduce chemically the free radical lesion thereby resulting in its repair^{1,2}. A similar mechanism has been postulated to explain the protective effect of promethazine against carbon tetrachloride-induced liver damage^{3,4}.

Although the scavenging actions of cysteamine (RSH) and promethazine (PZ) towards several electrophilic intermediates have been observed directly⁴⁻⁶, their reaction with nucleic acid related free radicals has not. Furthermore, it has been generally accepted that the initial products of reaction of the electrophilic hydroxyl radical with nucleic acid bases are adducts^{7,8}. We here report experiments which indicate that in the case of guanine derivatives, radical-cations are formed in high yield and these can react stoichiometrically with the above nucleophiles. The pulse radiolysis technique using both optical⁴⁻⁶ and conductometric⁹ analysing facilities has been employed.

On pulse radiolysis of nitrous oxide saturated solutions of either thymidine, cytidine, adenosine or guanosine (6×10^{-4} M,

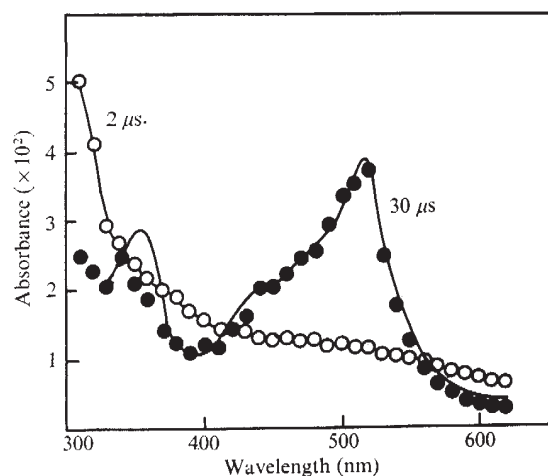


Fig. 2 Transient absorption changes observed on pulse radiolysis of nitrous oxide saturated solutions containing dGMP (10^{-2} M) and promethazine (10^{-4} M). O, Experimental points taken after 2 μ s; ●, experimental points taken after 30 μ s. The solid line through the 30 μ s points is the absorption spectrum previously assigned to the promethazine hydroxyl radical adduct (see text).

370 nm measured simultaneously shows identical decay kinetics and is therefore attributed to the radical-cation (R.L.W., and K.-D.A., unpublished).

In similar studies with slightly acid solutions of dGMP, radical cations were again detected. As in the case of guanosine the observable change in conductivity immediately after the radiation pulse, attributable to a reaction analogous to (1), decreased with increasing pH, no signal being observed at pH = 7. This does not, however, rule out the possibility of radical-cation formation in neutral solution. In the case of oxidised dGMP, in particular, the formation of a radical cation associated with a nitrogen atom and the simultaneous acid base dissociation of a phosphate residue would yield a zwitterion and would result in the absence of any accompanying change in conductivity. The fact that the transient optical absorption spectra of oxidised guanosine and dGMP were found to be similar in acid and neutral solution also provides support for the presence of similar species.

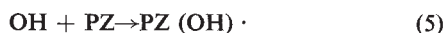
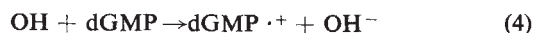
In the case of dGMP additional support for the formation of radical-cations is provided by studies with solutions containing excess bromide ions. Under the experimental conditions the electrophilic radicals $\text{Br}_2^{\cdot-}$ were formed rapidly. These decayed rapidly and exponentially. The associated absorption was replaced by an absorption similar to that attributed to the radical-cation in accord with the reaction:



$$k_3 = 4 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$$

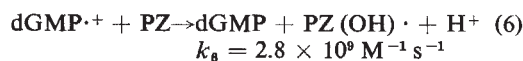
On pulse radiolysis of nitrous oxide saturated solutions of 5' dGMP (10^{-2} M , pH = 7) and promethazine (25–100 μM) the radical-cation absorption was again observed initially but decayed rapidly and was replaced by a new absorption, $\lambda_{\text{max}} = 520 \text{ nm}$ (Fig. 2). A similar absorption at 520 nm was observed with solutions of guanosine. With thymidine, however, no such absorption was observed in agreement with the lack of formation of long-lived radical-cations on reaction of the base with OH. In the case of dGMP the rate of formation of the new absorption, which was of similar shape and magnitude to that of the radical resulting from direct interaction of the hydroxyl radical with promethazine, increased with increasing promethazine concentration according to first order kinetics.

The absolute rate constants for the reaction:

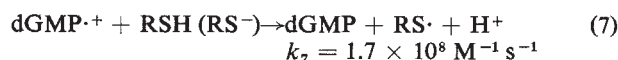


$$k_4 = 5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1} \text{ and } k_5 = 1.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$$

have been measured (R.L.W., and K.-D.A., unpublished and refs 10 and 11). As in the above experiment the concentration of dGMP $\gg$ PZ the appearance of the 520 nm absorption must be due to reaction (4) followed by:



Similarly on pulse radiolysis of solutions (pH 7.3) containing dGMP (10^{-2} M) and cysteamine (0.2–1 mM) the exponential decay of the nucleotide radical absorption observed at 580 nm is attributed to the reaction:



These results show clearly that the direct interaction between nucleophilic agents and free radicals produced in nucleic acid can occur rapidly. As these radicals are intermediates in lesion production, the above processes constitute a 'repair' mechanism. Furthermore, although these observations have concerned the

repair of lesions induced by the hydroxyl radical they could equally pertain to those induced by other electrophilic species such as those resulting from the biotransformation of carcinogenic or toxic agents, for example $\text{CCl}_3^{\cdot}$, $\text{RNOH}^{\cdot}$, or carbonium ions^{3,12}.

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Human tumours in mice confirmed by chromosomal analysis

THE growth of human tumours in mice may be evaluated by node size and histological sections¹⁻⁴. Because tumour cells may fuse with host cells⁵⁻⁷ *in vivo*, it is important to ascertain which cells produce the tumour. Human chromosomes may be identified using banding procedures and this makes it possible to verify that human cells alone grow in the tumour. If chromosomal analysis is performed on the tumour before injection, it would also be possible to determine which cells were able to grow rapidly *in vivo*. Here we present a report on such a chromosomal analysis of a human cervical carcinoma cell line grown in immunosuppressed mice and re-established *in vitro*.

Human cervical carcinoma cells were cultured from primary tissue by Sykes⁸. For this work they were cultivated in McCoy's medium with 20% foetal calf serum. The host animals were male 4–6 week old BALB/c/CRGL mice given 400, 500, or 600 rad of whole body X irradiation followed 2–3 h later with intravenous bone marrow cells from syngeneic donors. The mice were inoculated subcutaneously in the flank with 10^6 human cells in a total volume of 0.05 ml of culture medium without serum 24 h after irradiation. When a nodule was felt or when 21 d after irradiation was approaching, the animal was killed and a culture was initiated with minced tumour. Three independent tumours from mice of different irradiation doses were studied. For chromosome analysis, G bands were done with trypsin and C bands were produced using alkaline 2X SSC (ref. 9).

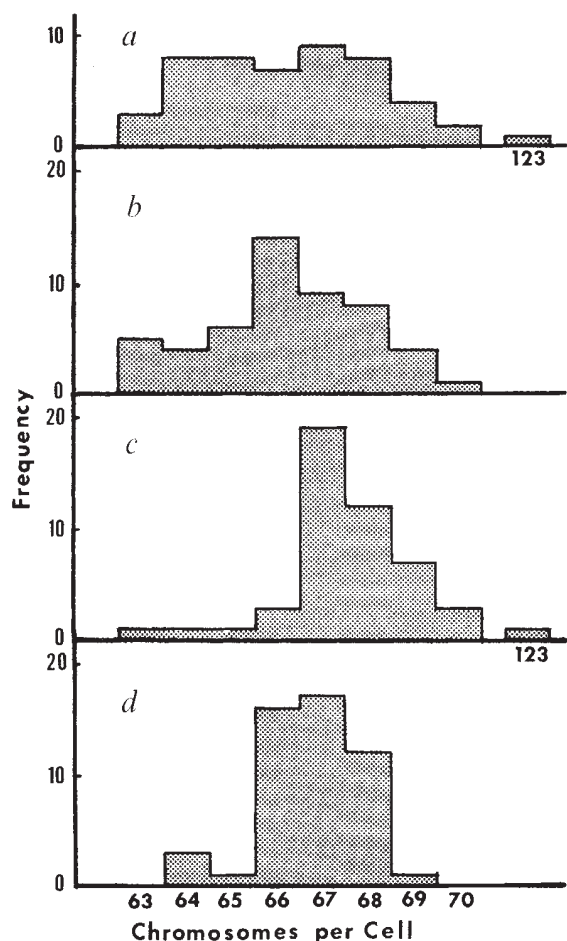


Fig. 1 Number of chromosomes counted in about 50 cells for each tumour. *a*, The original human cervical carcinoma cell line; *b-d*, cultures 1-3 (cell lines established from mouse-passaged tumours taken from three separate animals).

Within 3 weeks nodules could be felt in most of the mice. These nodules measured 1×2 mm to 4×7 mm. Some animals were allowed to survive, with regression starting at 28-30 d after irradiation. When cultures were started at 12-21 d, the tumour cells grew well in culture.

At the time this cell line was first described⁸, the chromosomal range of the cells was 48-130. At the time they were being grown for these experiments the cells had 63-70 chromosomes per cell with a mean of 66.1, a mode of 67 (18%), and 40% of these

cells having 66-68 chromosomes (Fig. 1*a*). After passage through mouse no. 1, culture 1 also had 63-70 chromosomes per cell with a mean of 65.6, a mode of 66 (28%) and 60% of the cells having 66-68 chromosomes (Fig. 1*b*). Culture 2 from mouse no. 2 had 63-70 chromosomes per cell with a mean of 67.5, a mode of 67 (38%) and 70% of the cells having 66-68 chromosomes (Fig. 1*c*). Culture 3 had a range of 64-69 chromosomes per cell with a mean of 66.7, a mode of 67 (34%), and 90% of the cells having 66-68 chromosomes (Fig. 1*d*). The cells with 66-68 chromosomes seemingly had the capacity to grow without restraint. There were very few cells with less than 66 or more than 68 chromosomes after transplantation. Fifty cells were analysed from each tumour.

The original carcinoma line was examined by G and C bands to determine the chromosomal constitution. The majority of the chromosomes could be identified with normal or near-normal trypsin-induced banding patterns (Fig. 2). There seemed to be representatives of each human chromosome present in each cell. In cells with fewer chromosomes the A 1 was frequently absent with non-random losses from other groups. Several new arrangements were seen that could not be specifically identified as originating from particular chromosomes, but C band patterns verified that they were not mouse chromosomes. Several very small metacentrics were observed—seemingly from the fusion of 21 and 22 long arms. At least one X chromosome could be identified in most of the cells.

The three cultures examined after mouse passage did not show a marked difference in karyotypes. There did not seem to be any particular chromosomes lost or gained, although the variation from cell to cell in such a tumour makes this very difficult to ascertain. The chromosomes were all human, no mouse chromosomes were seen at all. In the three independent tumours the mean and mode of the parental line was born out as the prime growth cells.

Human tumour biopsies are frequently cultured to examine the chromosomes, do growth kinetics, and assay drug responses. There are frequently several cell types that grow from such biopsies. The animal passage of such a mixed culture can select and concentrate the tumour-producing cells as an *in vivo* cloning procedure. In this study, cells with 66-68 chromosomes had properties allowing unrestricted growth, and could therefore be still considered malignant. Most important, the tumour in the mouse was shown to be composed of the human cells that had been injected.

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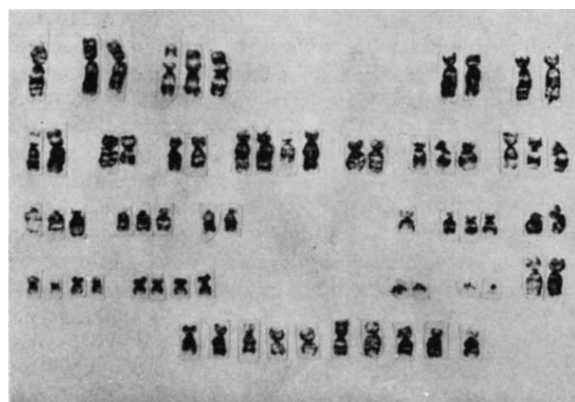


Fig. 2 Sykes tumour Giemsa-trypsin bands from a representative cell. Chromosomes were arranged according to normal and near-normal banding identification. The chromosomes on the last row showed too much rearrangement to allow positive classification.

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Metabolic activation of benzo(a)pyrene proceeds by a diol-epoxide

CARCINOGENIC polycyclic hydrocarbons such as the widespread environmental contaminant, benzo(a)pyrene (Fig. 1a), undoubtedly require metabolic activation. Boyland¹ proposed that epoxides, whose formation is catalysed by the NADPH-dependent microsomal mono-oxygenases^{2,3}, are the initial products of double-bond oxidation and we have suggested that epoxides are the important reactive metabolites responsible for the biological effects of these carcinogens⁴.

The properties of synthetic K-region epoxides lent some support to this hypothesis; they are alkylating agents that react covalently with nucleic acids, they are mutagenic in several systems and induce malignant transformation of rodent cells in culture⁵. Recent evidence suggests, however, that in cells treated with polycyclic hydrocarbons, it is not the K-region epoxides that react with DNA⁶.

Here we report that in cells in culture or in model systems *in vitro*, benzo(a)pyrene seems to undergo a two-stage metabolic activation that initially involves the formation of the

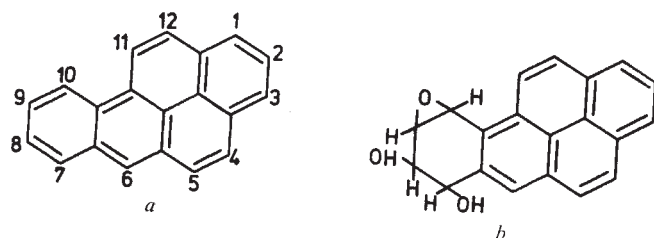


Fig. 1 Benzo(a)pyrene (a) and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide (b).

7,8-dihydrodiol⁷, which is then further metabolised on the isolated 9,10 double-bond by the microsomal mono-oxygenase to give 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide (Fig. 1b). Evidence that it is this type of diol-epoxide that actually reacts with DNA in cells treated with benzo(a)pyrene is also presented. As well as identifying, for the first time, the type of metabolite involved in the reactions of polycyclic hydrocarbons with genetic material, these findings also strongly support the general hypothesis that epoxides are ultimately responsible for the carcinogenicity of these compounds.

Primary cultures of Syrian hamster embryo cells were treated with benzo(a)pyrene labelled with either ³H or ¹⁴C. DNA was isolated from the cells, hydrolysed enzymically to nucleosides and the hydrolysates chromatographed on Sephadex LH20 columns eluted with methanol-water gradients^{6,8}. It is already known that nucleosides are eluted in the early fractions, for example Fig. 2, peaks I, II, IV and V; Fig. 3a peaks I, II and IV and Fig. 3c peaks I and II and that polycyclic hydrocarbon-nucleoside products elute later as the concentration of methanol in the eluting solvent increases^{6,8}. The elution profiles shown in Fig. 2 confirm that the DNA-products formed in benzo(a)pyrene-treated cells are different from those formed in reactions of the K-region epoxide, benzo(a)pyrene 4,5-oxide, with DNA.

As there was some evidence that the further metabolism of dihydrodiol metabolites might be involved in the reactions of

benzo(a)pyrene with DNA (ref. 9), ³H-labelled dihydrodiols were prepared in large-scale incubations of the radioactive hydrocarbon with rat-liver homogenate¹⁰ and incubated *in vitro* with a rat-liver microsomal preparation and DNA¹¹. The DNA was hydrolysed, mixed with a hydrolysate of DNA from cells treated with ¹⁴C-labelled benzo(a)pyrene and the mixtures chromatographed on Sephadex columns. Hydrolysates of DNA that had been incubated with microsomes and ³H-labelled 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene gave elution profiles in which the major hydrocarbon-deoxyribonucleoside product eluted in a position similar to that occupied by the DNA product from ¹⁴C-labelled benzo(a)pyrene treated

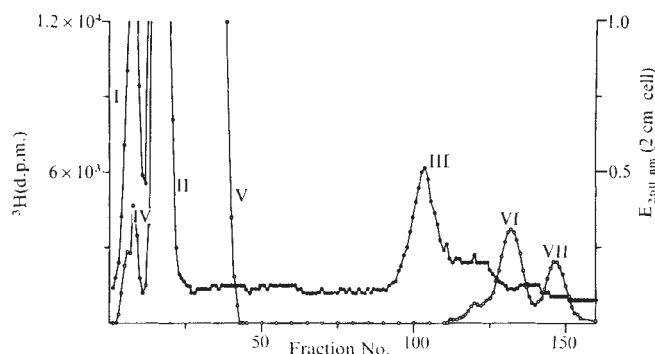


Fig. 2 Benzo(a)pyrene-deoxyribonucleoside products separated from hydrolysates of DNA by Sephadex LH20 column chromatography. ●, Hamster embryo cell DNA from cells treated with ³H-labelled benzo(a)pyrene; ○, DNA (salmon sperm) reacted with benzo(a)pyrene 4,5-oxide. The embryo cell DNA was obtained from primary cultures of Syrian hamster embryo cells grown in Dulbecco's MEM supplemented with foetal calf serum (15% v/v) in Thompson bottles (2 l) under 10% CO₂ in air. Confluent cell monolayers were treated for 24 h with ³H-labelled benzo(a)pyrene (1 µg ml⁻¹) (specific activity 3.24 Ci mmol⁻¹; the Radiochemical Centre, Amersham, UK) added as a solution in DMSO, final concentration 0.1%. Cells were collected with EDTA, the DNA isolated¹⁵ and hydrolysed with DNase followed by phosphodiesterase and alkaline phosphatase⁶. Salmon sperm DNA (Type III, Sigma Chemical Co., St Louis, Mo.) (50 mg) in Tris buffer pH 7.4, 0.01 M (50 ml) was mixed with benzo(a)pyrene 4,5-oxide (2.5 mg) in ethanol (25 ml) and incubated at 37°C for 4 h. The reaction mixture was extracted with ether (3 × 1 volume), the DNA precipitated with ethanol (1 volume), washed in ethanol and ether and dried⁴. Enzyme hydrolysates of this DNA and those of DNA from ¹⁴C-benzo(a)pyrene treated cells were cochromatographed on a column (75 × 1.5 cm) of Sephadex LH20 that was eluted with a methanol-water gradient^{6,8}. Fractions (5.3 ml) were collected and examined at 260 nm for ultraviolet absorbing materials; radioactivity was measured by liquid scintillation counting.

cells (Fig. 3a III and V). This finding was confirmed when DNA hydrolysates from embryo cells that had been treated with ³H-labelled 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene were compared with hydrolysates from ¹⁴C-labelled benzo(a)pyrene-treated cells (Fig. 3b, III and VI). Microsomal oxidation of the isolated 9,10-double bond present in the 7,8-diol of benzo(a)pyrene to give 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide seemed likely to be the second metabolic step, especially since reactions with DNA did not occur if the dihydrodiol was incubated with microsomal preparations in the absence of the cofactors necessary for the NADPH-dependent mono-oxygenase.

Accordingly ³H-labelled 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide was synthesised and reacted with DNA and a hydrolysate compared on a Sephadex column with a hydrolysate of DNA from cells treated with ¹⁴C-labelled benzo(a)pyrene. The elution profile (Fig. 3c) obtained from DNA reacted with the diol-epoxide contained two peaks of radioactivity in the region normally occupied by hydrocarbon-deoxyribonucleoside products, one of which (Fig. 3c, III) was

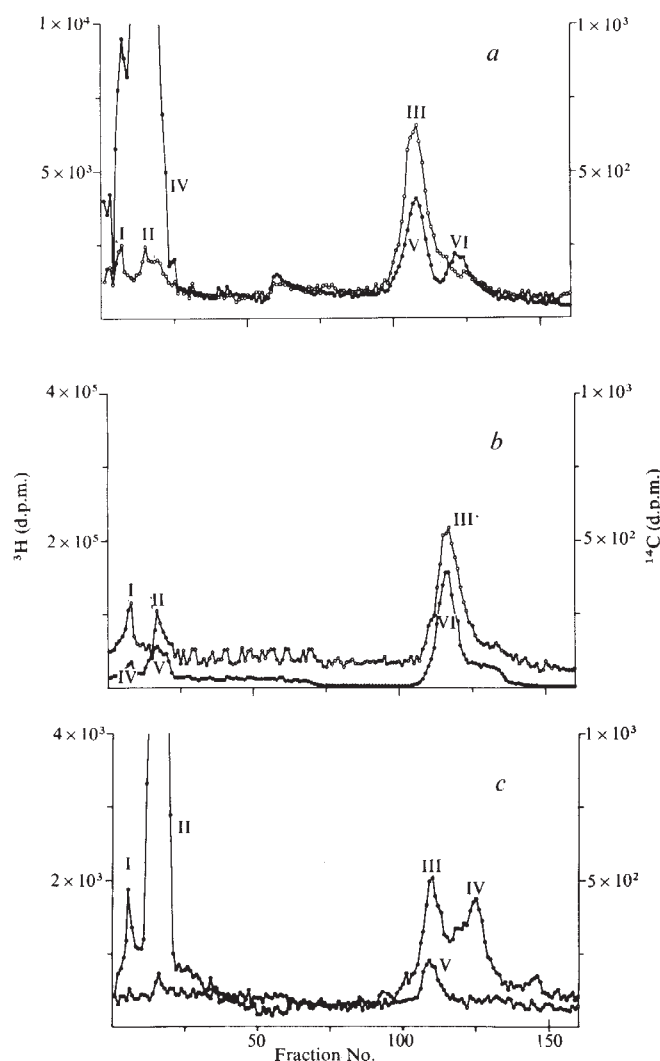


Fig. 3 Benzo(a)pyrene-deoxyribonucleoside products separated from hydrolysates of DNA by Sephadex LH20 column chromatography. *a*, ○, DNA from hamster embryo cells treated with ^{14}C -benzo(a)pyrene (specific activity 21 mCi mmol^{-1}). DNA was isolated and hydrolysed as described in the legend to Fig. 1. ●, DNA incubated with ^3H -7,8-dihydro-7,8-dihydroxybenzo(a)pyrene in the presence of a rat liver microsomal preparation. DNA (salmon sperm) (60 mg) in Tris buffer pH 7.4, 0.01 M, (40 ml) was mixed with a washed rat liver microsomal preparation¹¹ ($\approx 5 \text{ g liver}$) resuspended in Tris buffer (40 ml) containing NADPH (24 mg), glucose 6-phosphate (120 mg) and glucose 6-phosphate dehydrogenase (14 units) to which ^3H -7,8-dihydro-7,8-dihydroxybenzo(a)pyrene (specific activity $437 \text{ mCi mmol}^{-1}$) ($43 \mu\text{g}$) was added in acetone (0.2 ml). After incubation for 30 min at 37° , the microsomes were pelleted by centrifugation and the DNA reisolated and purified¹¹. Hydrolysates of this DNA were cochromatographed with those from ^{14}C -benzo(a)pyrene-treated cells. *b*, ○, DNA, from hamster embryo cells treated with ^{14}C -benzo(a)pyrene, hydrolysed and cochromatographed with an hydrolysate of ●, DNA from hamster embryo cells treated similarly with ^3H -7,8-dihydro-7,8-dihydroxybenzo(a)pyrene (specific activity $3.24 \text{ Ci mmol}^{-1}$) ($0.25 \mu\text{g per ml medium}$). *c*, ○, DNA from hamster embryo cells treated with ^{14}C -benzo(a)pyrene hydrolysed and cochromatographed with an hydrolysate of ●, DNA reacted with ^3H -7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide. The diol-epoxide was synthesised by treating the radioactive 7,8-dihydrodiol ($235 \mu\text{g}$) with *m*-chloroperoxybenzoic acid ($200 \mu\text{g}$) in chloroform (2 ml) at 0°C for 48 h. After washing with NaOH and water, the chloroform solution was evaporated to dryness, the residue dissolved in ethanol and the epoxide purified by thin-layer chromatography on an Eastman-Kodak 6060 Chromogram developed with cyclohexane:dioxan, 1:1 (v/v). The diol-epoxide (specific activity $437 \text{ mCi mmol}^{-1}$) ($100 \mu\text{g}$) in ethanol (1.5 ml) was mixed with DNA (salmon sperm) (3 mg) in Tris buffer (3 ml) and incubated, and the DNA reisolated and hydrolysed (see legend to Fig. 1).

coincident with the DNA product from ^{14}C -labelled benzo(a)pyrene-treated cells: the second product (Fig. 3c, IV) has not been further investigated. Small amounts of this second product seem to be formed when the 7,8-diol is incubated with microsomes and DNA (Fig. 3a, VI) but were not seen when either benzo(a)pyrene or the 7,8-diol were metabolised by embryo cells. The ^{14}C - and the ^3H -labelled hydrocarbon-deoxyribonucleoside products that eluted together from Sephadex columns (Fig. 3a, III and V; Fig. 3b, III and VI; Fig. 3c, III and V) were further examined on thin-layer chromatograms. Three dissimilar solvent systems failed to separate the ^{14}C -labelled benzo(a)pyrene-deoxyribonucleoside products obtained from cells treated with the hydrocarbon from the ^3H -labelled benzo(a)pyrene-deoxyribonucleoside products formed in the other experiments; in each case the ^{14}C and the ^3H -labelled products migrated together.

In other experiments, ^3H -labelled samples of synthetically-prepared 4,5- and metabolically-obtained 9,10-dihydrodiols of benzo(a)pyrene, which are also metabolites of this hydrocarbon⁷, were used. Hydrolysates of DNA from embryo cells treated with the 4,5- and 9,10-dihydrodiols did not appear to contain radioactive hydrocarbon-deoxyribonucleoside products although some were formed from the 9,10-derivative in microsomal incubations. Attempts to prepare 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene 7,8-oxide were not successful; the 9,10-double bond of benzo(a)pyrene seems to be chemically much less reactive than that in the 7,8-position.

Our results indicate that an isolated double-bond in the 9,10-position of benzo(a)pyrene leads to the metabolic formation of a 9,10-epoxide and that an epoxide formed in this position reacts effectively with cellular DNA. The dihydrodiols that are precursors of this type of epoxide are common as hydrocarbon metabolites and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene has been detected as a benzo(a)pyrene metabolite that is formed by rodent liver and lung^{7,9} and by human lymphocytes¹². Preliminary experiments with other hydrocarbons imply that analogous metabolic activation mechanisms also apply, for example, to benz(a)anthracene and to 7-methylbenz(a)anthracene. They also indicate that diol-epoxides of the type discussed here may be effective in reaching cellular DNA simply because diol-epoxides are not good substrates either for the epoxide hydrolase¹³ or for the glutathione transferase¹⁴, enzymes that normally inactivate epoxides. The work carried out so far indicates that diol-epoxides like 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide are important in the metabolic activation of the polycyclic hydrocarbons to intermediates that react with DNA; consequently they might be expected to be biologically active and appropriate tests for carcinogenicity and mutagenicity are in progress.

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Babesia microti and *Plasmodium berghei yoelii* infections in nude mice

THE precise nature of the responses to *Babesia* spp. and *Plasmodium* spp. which lead to their elimination from the host and subsequent acquisition of immunity is uncertain. A degree of thymus dependence is involved, as shown by the aggravation of infection by neonatal thymectomy^{1,2}, anti-thymocyte serum³ and anti-lymphocyte serum^{4,5}. For instance, parasitaemias due to *P. berghei* were higher in neonatally thymectomised rats than in controls, and lasted about twice as long, with higher mortality^{1,2}.

The recent availability of congenitally hypothyroid (nude *nu/nu*) mice allows a more critical appraisal of the importance of the role of thymus competence in the elimination of these parasites. *B. microti* and *P. berghei yoelii* produce transient parasitaemias in normal mice, followed by recovery and resistance to challenge with the same species of parasite^{6,7}. We have investigated these infections in nude mice.

Groups of three nude mice or normal littermates 6-8 weeks of age were infected by intraperitoneal inoculation with 10⁶ erythrocytes parasitised by either *B. microti* (King's 67 strain, provided by Dr F. E. G. Cox) or *P. berghei yoelii* (17 × strain, from Dr N. Wedderburn). The nude mice were housed in germ-free isolators. Tail blood smears were stained with Giemsa, and the number of parasitised cells in 400 erythrocytes counted. The course of the *B. microti* infection is summarised in Fig. 1a, and of *P. berghei yoelii* in Fig. 1b. Means and ranges are indicated.

Following a latent period that was the same in all cases, there was a transient parasitaemia in littermate control mice and a persistent parasitaemia in nude mice. Apparent differences in the nature of this persistence of each parasite can be explained in terms of preference for different developmental stages of the red cell series, for whereas *B. microti* seems largely to be restricted to mature red cells *P. berghei yoelii* parasitises only reticulocytes. Nearly all the reticulocytes in the *P. berghei yoelii* infected nude mice contained parasites from day 10. Thus the rise in reticulocyte number with worsening anaemia, rather than any difference in the response of nude mice to the two organisms, is likely to be responsible for the overall parasitaemia remaining stable with one (Fig. 1a) and increasing with the other (Fig. 1b). A high and stable percentage of the susceptible red cell stage was infected by each organism until the nude mice eventually died or were killed.

In contrast to our findings, neonatally thymectomised rats took only twice as long as normal littermates to clear *P. berghei* from the peripheral circulation^{1,2}. There is evidence that neonatal thymectomy in rats does not completely eliminate T lymphocytes⁸. Thus, in keeping with data obtained with anti-thymocyte serum³, the thymus dependence of elimination of *Plasmodium* spp. from rodents appears to be more complete than earlier work suggests.

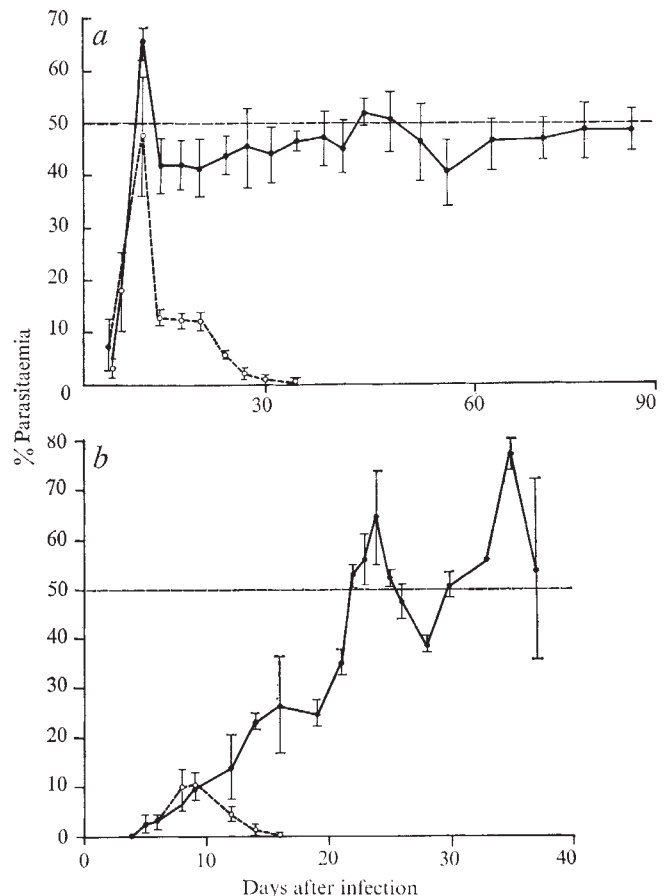


Fig. 1 a, *Babesia microti* infection in nude mice and normal littermates. b, *Plasmodium berghei yoelii* infection in nude mice and normal littermates. ●, *nu/nu*; ○, *nu/+*.

The results obtained with *B. microti* in nude mice (Fig. 1a) were similar to those found on giving anti-lymphocyte serum to hamsters before and during infection with this parasite⁶. This confirms an absolute dependence on the thymus for elimination of this parasite as well as *P. berghei yoelii*. Nude mice may be a more suitable model because they seem to survive persistent parasitaemias of *B. microti* more successfully than do anti-lymphocyte serum treated hamsters. On day 80, twice as long as any hamster survived, nude mice were apparently quite healthy after maintaining a 50% parasitaemia for the previous 70 d. In addition the problem of anti-lymphocyte serum standardisation inherent in the hamster model is eliminated.

In summary, the present data confirm that elimination of *B. microti* and *P. berghei yoelii* from the circulation of mice does not occur without thymus competence. It remains to be established whether the thymus-derived cells exert a helper effect in the production of a particular class or subclass of antibody required for parasite elimination, or form part of another mechanism of immunity. Further experiments are in progress to test these possibilities. Clearly the parasitised nude mouse could prove to be a useful model for further immunological research in this field.

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Evidence for oligomeric IgA production by peripheral rat lymph nodes

A DISTINCTIVE characteristic of IgA is its polymorphic nature with regard to the molecular size. It occurs in secretions mostly as a dimer or tetramer^{1,2} while serum IgA has a polydisperse weight distribution in many species¹. It is mostly monomeric in human serum (7S) (ref. 3), mostly polymeric in the mouse⁴ and in the dog⁵, but in both these and other species several molecular sizes occur in the blood¹.

When extending studies on natural anti-hapten antibodies in the rat⁶ we made the unexpected finding that natural anti-3-iodo-4-hydroxy-5-nitrophenylacetyl (NIP) of the thoracic duct lymph had sedimentation characteristics (9S-13S) similar to the secretory IgA antibodies that we could demonstrate in rabbit colostrum⁷ and to the oligomeric IgA produced by mouse spleen cultures⁸. Natural antibody in the blood serum of the same rats sedimented like 19S IgM. This finding prompted us to study sedimentation patterns of anti-NIP antibodies in the blood and the lymph of immunised rats.

Rats were immunised with 10 µg NIP-SIII pneumococcal polysaccharide⁸ or 500 µg of NIP chicken globulin (CG)⁸ in complete Freund's adjuvant (CFA). As we wanted the antibody production to take place preferentially in the peripheral lymph nodes draining to the thoracic duct the antigen was injected into the hind foot pads. The rats were bled and cannulated (abdominal thoracic duct) 8-9 d after immunisation. Thoracic duct lymph samples were clarified by spinning for one hour at 50,000g. The lymph and the serum samples were titrated with NIP-T₄ bacteriophages as described earlier⁹. They were centrifuged through a continuous and linear 5-20% sucrose gradient in 12 ml tubes in rotor SW-41 Ti of a Beckman L 3-50 ultracentrifuge for 14 h (39,000 r.p.m.). A total of 25 fractions were collected and titrated with NIP-T₄. Sedimentation constants (*S*_{20,w}) were derived according to McEwen¹⁰.

The predominant antibody peaks of the blood serum had sedimentation constants of 19S and 7S. A minor 9S component could usually be detected (Fig. 1a). Antibodies of the thoracic duct had major 19S and 7S fractions but they also had considerable 13S and 11S fractions (Fig. 1b); 9S antibody could be seen as a distinct peak in some but not all lymph samples. We have found this phenomenon in all the 14 serum-lymph pairs studied so far.

In some experiments we incubated diluted lymph before centrifugation with rabbit anti-rat IgA. Anti-IgA is known to

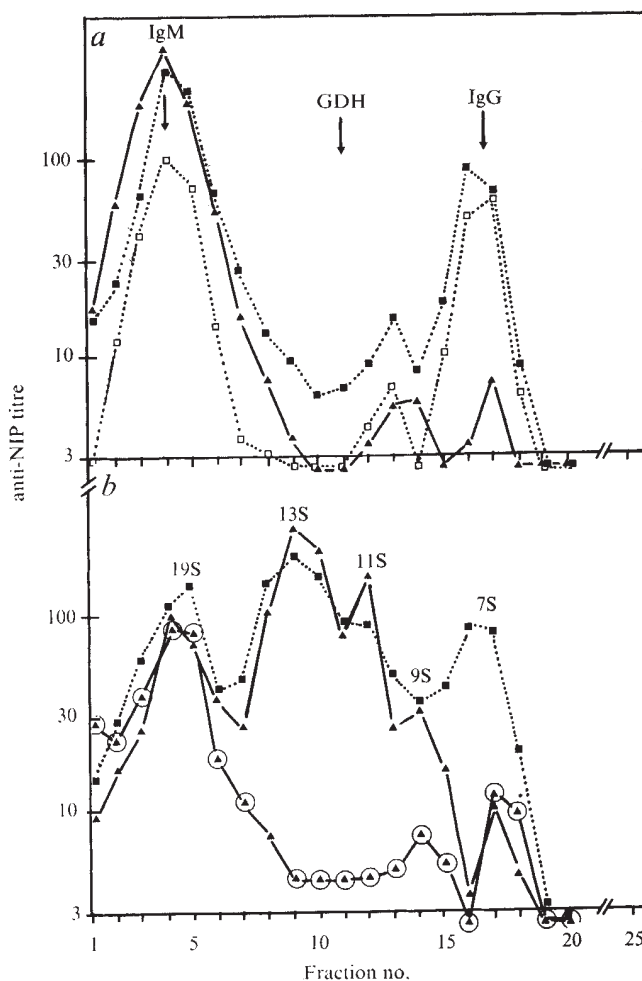


Fig. 1 Sedimentation pattern of anti-NIP, *a*, in the serum and *b*, thoracic duct lymph of rats after immunisation with NIP-CG and NIP-SIII. —, NIP-CG immunisation; . . . , NIP-SIII immunisation; encircled triangles, sample mixed with anti-IgA. Different symbols indicate different rats. Rabbit anti-DNP and glutamate dehydrogenase (GDH) were used as reference compounds, their maximum activity is indicated by arrows.

displace or eliminate IgA but not IgG or IgM antibodies in the sedimentation pattern⁸. Data in Fig. 1 show that the 9S, 11S and 13S peaks were displaced by our anti-IgA and were thus very probably IgA.

The total IgA of several lymphs and sera was determined by the method of Mancini, Carbonara and Heremans¹¹. We found that concentrations in the lymph fluid were 4-12 times higher than in the serum of the same animal. In the ultracentrifugation the IgA of the lymph fluids was fairly evenly distributed from the 7S to 15S fractions while the serum IgA was distributed from the 7S to 11S fractions.

The Stokes's radii of the four heaviest antibody fractions were determined by gel filtration according to Laurent and Killander¹². They were calculated as means of two gel filtration runs, through a Sepharose 6B column (1.5 × 79 cm). Reference proteins in these runs included rat serum albumin, hyperimmune rabbit anti-DNP (IgG) and glutamate dehydrogenase. Their radii were assumed to be 35, 51 and 60 Å respectively^{13,14}. The fractions from the gel filtration were rerun in ultracentrifugation. Molecular weights (Table 1) were calculated using the *S*_{20,w} values and the Stokes radii in the formula

$$M = (6\pi\eta_{20,w}Na_{20,w}) / (1 - \bar{v}\rho_{20,w})$$

in which $\eta_{20,w}$ and $\rho_{20,w}$ are the viscosity and density of water at 20° C, N is Avogadro's constant and a is Stokes's radius. The

Table 1 Characteristics of the antibody classes that could be separated from the thoracic duct of immunised rats and lymph node culture fluids

Sedimentation constant	Estimated Stokes' radius (Å)	Ig class	Determined molecular weight	Molecular weight of the theoretical human IgA polymer that would come closest to the observed molecular weight (see text)
19S	107	IgM	860,000	
13S	91	IgA	515,000	520,000
11S	80	IgA	380,000	370,000
9.4S	70	IgA	280,000	320,000

partial specific volume ($\bar{v}$) of oligomeric IgA was assumed to be 0.73 (ref. 15).

It is possible that 9S-13S IgA anti-NIP of the thoracic duct lymph mainly originates in the intestinal lymphoid tissue. For instance, plasmablasts generated in the regional lymph nodes may be transferred to the intestinal area and produce antibody there¹⁶. It was made unlikely, however, by the finding that when popliteal lymph nodes were removed from these rats and kept in organ cultures for 6 h without additional antigen they produced the same 9S-13S fractions which we found in the thoracic duct lymph. These again could be displaced by anti-IgA (Fig. 2).

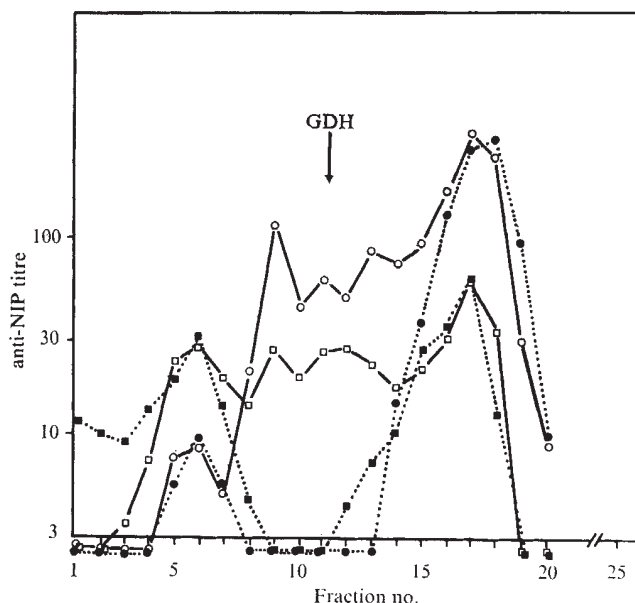


Fig. 2 Sedimentation pattern of anti-NIP activity produced by two organ cultures. Popliteal lymph nodes were removed 6 or 9 d after a foot pad immunisation (NIP-SIII) and 1 μ l pieces were cultured⁸ for 6 h ($\square$, $\blacksquare$) or 24 h ($\circ$, $\bullet$) without additional antigen. $\square$ and $\circ$, untreated samples; $\blacksquare$ and $\bullet$, samples mixed with anti-IgA, the rat represented by open squares is represented in the same way in Fig. 1.

Data in Fig. 2 are derived from the first two lymph node cultures we tested but we have studied five nodes from five rats. Four nodes produced the 9S-13S IgA antibody. One node produced little antibody, and all of it was 19S. These data suggest that at least some 9S-13S IgA anti-NIP was produced by regional lymph nodes of our rats.

We considered the possibility that the polymerisation had taken place outside the synthesising organs. The following facts, however, fail to support it. First, individual lymph samples maintained their sedimentation pattern through weeks of storage at -20°C . Second, centrifugation of lymph 15 min after it had left the thoracic duct showed a similar sedimentation pattern to lymph samples centrifuged later (rat no. 5 marked with closed squares in Fig. 1b). Third, *in vitro* synthesis of 9S-13S IgA antibody by lymph node fragments.

We believe that the different IgA antibody fractions represent different degrees of polymerisation. The molecular formulae of the three IgA fractions cannot be derived from our data but if we make the assumption that the molecular weight of the light chain, the α chain, J piece, secretory piece, and the carbohydrate content are the same in the rat IgA as those in man we find that our observed molecular weights would correspond reasonably well with the theoretical molecular weights of the following reconstructed human polymers; trimer+secretory piece+J piece (our 13S IgA), a dimer+secretory piece+J piece (our 11S IgA) and dimer+J piece (our 9S IgA).

We conclude that non-intestinal lymphoid tissues produce

polymerised IgA antibodies in immunised rats. This antibody can be demonstrated in the thoracic duct lymph and cultures of popliteal lymph nodes. It occurs in three main size classes with molecular weights of approximately 515,000, 380,000 and 280,000. In the blood these antibodies, especially the two heavier classes, are either rapidly catabolised, reduced to small subunits, or secreted.

We thank Dr Ari Helenius for his advice and help in the determination of molecular weights and Mrs Maire Laakso for the cannulation of thoracic ducts. This work was supported by the Medical Research Council, Academy of Finland.

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Antigenic inhibition of cell-mediated cytotoxicity against tumour cells

THERE is now abundant evidence that tumour cells express new surface antigens (tumour-associated antigens, TAA)¹. In many tumour models, cell-mediated immunity directed against TAA has been demonstrated *in vitro*². Serum from tumour-bearing individuals has been shown to inhibit specifically cell-mediated immune responses (CMI) to the tumour target cells *in vitro*³, and this phenomenon may explain the paradoxical coexistence of a growing tumour and an immune response directed against the tumour. Both immune complexes of TAA with antibody^{4,5}, and soluble TAA alone⁶, have been implicated as inhibitors in these sera.

In most studies, however, neither the nature of the effector cell nor the mechanism of inhibition, have been well characterised. CMI against tumours induced in mice by murine sarcoma virus (MuSV), which was measured in a microcytotoxicity assay (MCA), and in which both T and non-T effector cells were cytotoxic, was inhibited by progressor serum and by soluble TAA⁷. In concurrent studies with a short term ⁵¹Cr release assay, in which only T cells were cytotoxic, there was no inhibition. In antiallogeneic CMI, cytotoxicity by T cells

Table 1 Effect of tumour-bearer sera on CMI

Origin of Serum		% Inhibition of specific lysis*
Hyperimmune animals	—8	(15.2/14.1)
Regressor animals	5	(22.0/23.0)
	4	(13.5/14.1)
	14	(12.3/14.1)
	17	(11.7/14.1)
Progressor animals		
Intact	30	(9.9/14.1)
	36	(9.1/14.1)
	47	(7.5/14.1)
	48	(7.3/14.1)
	83	(2.3/14.1)
Immunosuppressed	800 r.	52 (11.0/23.0)
	800 r.	35 (15.0/23.0)
Anti-lymphocyte globulin	36	(14.7/23.0)

5×10^6 spleen cells from 10 d immune rats in cold RPMI-1640 supplemented with HEPES buffer (20 mM) and 10% heat inactivated foetal calf serum, were added to 5×10^4 ^{51}Cr -labelled W/FuG-1 lymphoma cells (an *in vitro* adapted line), in the 16 mm wells of Linbro trays. After 4.5 h at 37°C with rocking (six to and fro tilts through 15° per min), ^{51}Cr release was determined and the specific release calculated¹⁰. Controls included spleen cells from age-matched normal rats and target cells incubated alone. Maximum releasable counts were those released following the incubation of target cells in 1.5% Brij detergent.

% Specific release of $^{51}\text{Cr} = \frac{I \text{ c.p.m.} - N \text{ c.p.m.}}{(\text{Detergent c.p.m.} - N \text{ c.p.m.})} \times 100\%$ where I and N are the counts released by immune and normal cells. Spontaneous release was usually 15–20% and the release by normal cells, 25–35% of the detergent release respectively. % inhibition = $\frac{I - I_{\text{inhib}} \times 100}{I}$, where I and I_{inhib} are the % specific release by

immune cells in the absence or presence of inhibitor respectively. Sera, including normal rat serum used as a control, were heat inactivated and added at a final concentration of 1:5 to lymphocytes and target cells, and to target cells alone. Irradiation was administered from a ^{60}Co source. Anti lymphocyte globulin was made in pigs to rat thymocytes. Rats received 50 mg subcutaneously on days –4 and 0.

*Immune cells + inhibitor/immune cells only.

could⁸ or could not⁹ be inhibited by soluble fractions containing H-2 antigen.

The syngeneic transplantable Gross virus-induced lymphomas (C58NT)D and W/FuG-1 are rejected after subcutaneous injection within 10–15 d in about 85% of adult W/Fu rats¹⁰. Progressive, lethal tumour growth is observed following intraperitoneal injection of (C58NT)D cells, when the tumour grows in ascitic form, or following the subcutaneous injection of either tumour in immunosuppressed rats. The CMI response of rats to these tumours, as measured in a ^{51}Cr -release assay, is mediated by T cells^{10–12}. Here we report the inhibition of cytotoxicity by progressor sera and also by cell-free TAA.

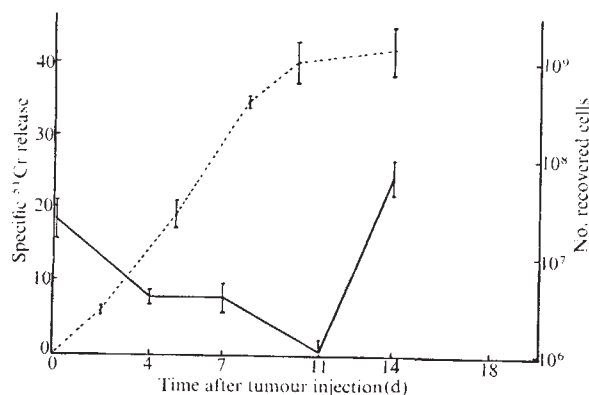


Fig. 1 Effect of progressor serum on CMI. Sera taken at various times from a group of six rats injected with 10^6 (C58NT)D cells intraperitoneally, were tested at a dilution of 1:5, for inhibition of CMI with 10 d immune spleen cells and ^{51}Cr -labelled W/FuG-1 cells (cell ratio 100:1). All other experimental conditions were as described in Table 1. —, % specific ^{51}Cr release; - - -, No. recovered cells. Standard errors are indicated.

All inhibitors were tested in a 4.5 h ^{51}Cr release assay using immune spleen cells taken 10 d following the subcutaneously injection of 10^6 (C58NT)D cells into adult inbred W/Fu rats^{10,13}.

In these experiments progressive growth of tumours was obtained following the subcutaneous injection of 10^6 W/FuG-1 cells into immunosuppressed rats, and occasionally in normal rats. Tumour diameters were measured regularly to define the progressive and regressive phases of growth.

In Table 1, hyperimmune serum (raised by multiple injection of 10^7 W/FuG-1 lymphoma cells) is compared with progressor and regressor sera from the five adult rats, out of an injected group of 31, which showed tumour growth beyond 20 d. Progressor sera were taken during the phase of progressive growth between 20 and 30 d and regressor sera were taken from the same rats during regression of the tumours. Progressor sera from immunosuppressed rats were taken at 14–20 d or just before death.

Table 2 Inhibition of CMI by TAA

Antigen	% Inhibition of specific lysis dose of antigen (µg)					
	400	100	50	30	10	3
(C58NT)D extract*	73	42		39	22	
MuLV-G		54	39	31	9	3
MuLV-G F/T†			88			
MuLV-M				31		
MuSV-M		44			14	
MuLV-G formal‡		31		19	11	9
p31		47				

Immune spleen cells (5×10^6) were incubated with antigens for the duration of the assay. The antigens were added in 0.1 ml volumes and were also tested on target cells only. As a control, an equivalent volume of PBS was added to both lymphocytes and target cells, and to target cells only. The Gross and Moloney pseudotypes of murine leukaemia virus, MuLV (MuLV-G and MuLV-M) were purified by differential centrifugation from *in vitro* culture supernatants of W/Fu Gross and Moloney virus-induced lymphomas. MuSV-M was obtained from cultures of a BALB/c mouse sarcoma line (MSC)¹⁷. p31 was prepared from MuSV-M by an isoelectric focusing method¹⁸. Crude soluble extracts were made of (C58NT)D cells and normal rat spleen cells by papain digestion¹⁹. All reagents were dialysed exhaustively against phosphate buffered saline (PBS) before use. The results are the means of several experiments. All reagents in Tables 2 and 3 were tested at least twice and mostly three to four times. In no case was the standard error of the mean % inhibition greater than 5%. The mean of the % specific cytotoxicity, without added inhibitors, in these experiments was $28 \pm 3\%$ s.e. Antigen concentrations were calculated by the method of Lowry²³ using bovine serum albumin as standard.

*Crude soluble fraction of a papain digest.

†Eight freeze-thaw cycles.

‡Formalised virus, 0.01% formalin, 4°C for 2 weeks (ref. 20).

Statistically significant ($P \leq 0.01$) inhibition of CMI is seen with all progressor sera by comparison with regressor sera. Normal rat serum itself exerts an apparent small inhibitory effect, largely due to the lower spontaneous release of ^{51}Cr in the presence of rat serum. When sera were tested at dilutions up to 1:10,000, augmentation of CMI was observed with hyperimmune and regressor sera, but not with progressor sera. The augmentation may reflect cytotoxicity by non-immune cells in the presence of an antibody in hyperimmune and regressor sera (lymphocyte dependent antibody)¹⁴ which, characteristically, is effective at high dilution¹⁵. The inhibitory effect of progressor sera was lost at dilutions greater than 1:20. Where sequential bleeds were taken during progression or regression of the tumour, the % inhibition of CMI was directly related to tumour diameter.

In Fig. 1, sera from adult rats with a progressive ascites lymphoma (10^6 (C58NT)D cells injected intraperitoneally) were tested for inhibition of cytotoxicity at various times during tumour growth. The rats died at a mean of 14.7 d. Tumour growth was measured by the number of cells recovered from the peritoneal cavity. Increasing inhibition of CMI by serum

on days 4, 7 and 11 ($P \leq 0.01$) is seen during the tumour growth, but inhibition is lost just before death when tumour growth rate has decreased. The characterisation of the inhibitor in serum is in progress.

To test the effect of cell-free antigen alone on CMI, whole virus, the major viral group specific antigen p31 (ref. 16), and a soluble papain digest of tumour membrane, were used.

The results (Table 2) show that equal amounts of both the Gross and Moloney pseudotypes of MuLV, and formalin-treated non-infectious MuLV-G, produce approximately the same inhibition of CMI. The inhibition by formalin-inactivated virus suggests that inhibition does not result from viral infection of effector lymphocytes. The group specific inhibition by virus may reflect a degree of particle disruption during virus purification with release of the internal group-specific antigens. The most abundant of these, p31, has been detected in virus stocks (R.A.K., unpublished). Furthermore, an aliquot of MuLV-G intentionally disrupted by eight freeze-thaw cycles is 2.3 times as potent as the same undisrupted virus stock (89% against 39% inhibition—Table 2). Purified p31 itself inhibits cytotoxicity.

Table 3 Specificity of inhibition

a CMI to (C58NT)D* Antigen		% Inhibition of specific lysis dose of antigen (μ g)					
		1000	400	100	30	10	3
Normal spleen extract			6	3			
Bovine serum albumin		1		2		3	1
Newcastle Disease Virus			—6	—5		—4	2
MuLV-G				54	31	9	3
b CMI to L5178Y†		Added antigen (μ g)		% Specific lysis			
Ratio							
200:1				49.5			
100:1				16.0			
50:1				4.6			
100:1	MuLV-G 100			15.7			
100:1	MuSV-M 200			16.3			
100:1	MuSV-M 100			16.7			
100:1	(C58NT)D extract 300			15.9			
100:1	(C58NT)D extract 150			14.9			

*CMI performed with 5×10^6 lymphocytes (that is, ratio of 100:1).

†Normal or immune spleen cells from W/Fu rats immunised 7 d previously with 2×10^8 murine L5178Y lymphoma cells intraperitoneally, were assayed with 5×10^4 ^{51}Cr -labelled L5178Y cells for 8 h at 37°C with rocking. The results of a single typical experiment are shown.

T lymphocytes, purified from L5178Y immune rat spleen cells by nylon column fractionation, are cytotoxic for ^{51}Cr -labelled L5178Y lymphoma cells. At a ratio of 100:1 the specific cytotoxicity of fractionated cells was 12.7%, and that of the unfractionated cells was 7.1%, in a typical experiment.

The inhibition by virus and viral antigens is specific (Table 3a). It can be seen that an antigenically unrelated virus, Newcastle Disease Virus, and soluble antigens from normal rat spleen cells do not inhibit CMI in comparison with MuLV-G. A syngeneic CMI response in the rat directed against TAA induced by an unrelated virus and measured in a short term ^{51}Cr release assay, would have been the ideal control for the specificity of inhibition of CMI. In the absence of such a system, MuLV-G, MuSV-M and (C58NT)D extract were tested in an anti-xenogeneic system (Table 3b). The spleen cells of rats immunised intraperitoneally with 2×10^8 murine L5178Y lymphoma cells 7 d previously, exhibit marked CMI towards ^{51}Cr -labelled L5178Y cells in an 8 h assay, but this response was not inhibited by the added TAA.

Preliminary experiments suggest that the inhibition of CMI by cell-free TAA is at the level of the effector cell. The cytotoxicity of spleen cells was inhibited by 30% following preincubation with MuSV-M before assay (100 μ g per 5×10^6 cells). Preincubation of target cells with antigen did not alter the cytotoxicity.

These experiments were performed with unfractionated

immune spleen cells, in which T cells have been shown to be the effector cells^{10–12}. To substantiate the direct inhibition of T effector cells by antigen, B cells and macrophages were removed by incubation of immune spleen cells on nylon wool columns²¹. Phagocytic cells are regularly reduced from 7–9% of total cells to about 0.5% by this procedure¹⁰. The specific cytotoxicity by column purified cells at a ratio of 100:1 (in which the percentage of immunoglobulin bearing cells had been reduced from 40% to 4%) was 35%, and was inhibited by MuSV-M virus disrupted by freeze-thawing (40 μ g produced 35% specific inhibition), and by a papain digest of (C58NT)D lymphoma cells (350 μ g inhibited 96%). The same spleen cell population, tested without fractionation, was also inhibited by the same dose of these antigens.

Our evidence indicates that effector T cells can be inhibited in a ^{51}Cr release assay by progressor sera and by cell-free TAA. This differs from results obtained in the MuSV tumour model in mice where CMI was inhibited by TAA and progressor serum only in MCA, in which both T and non-T cells were cytotoxic⁷, and not in a ^{51}Cr release assay. This may reflect species differences in functional accessibility of receptors to the antigens studied. Our results indicate that the expression of a cell-mediated immune response can be inhibited by antigen at the level of the final effector cell. A recent report²² indicates that antigen can also inhibit the release of antibody from antibody forming cells.

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reviews

The Interrelationships of Fishes is a very welcome volume, for it contains an up to date statement of position by leading authorities on nearly every major group of fishes and, therefore, it provides an almost comprehensive survey of the state of palaeoichthyology in 1972. Tipping the scales at three and a half pounds it is in every sense a heavyweight volume, but its publishers and editors are to be congratulated on an attractive format and a text that is almost free of blemishes.

The interrelationships of most of the groups of fish have long been uncertain. That is partly because most major groups appear suddenly, already fully distinct, in the Devonian, and partly because of the rapid and complex evolution that characterised the emergence and early evolution of the teleosts. Though some new discoveries, and even more new opinions, have appeared in the last few years, it has been difficult for the non-ichthyologist to gauge the extent to which these have been accepted, especially in their implications for the major dichotomies in the evolution of fishes.

'Dichotomies' is the obligatory term, for the procedures of phyletic analysis advocated by Hennig have penetrated more thoroughly into the world of palaeoichthyologists than into other fields of vertebrate palaeontology, and the book is notable for its uniformity of approach in such matters. Irrespective of the merits or demerits of these methods, the uniformity does make it easier to analyse the precise points at issue where opinions differ.

Fish families

Barry Cox

Interrelationships of Fishes. Edited by P. H. Greenwood, R. S. Miles, and Colin Patterson. (Supplement No. 1 to the Zoological Journal of the Linnean Society.) Pp. xiv+536. (Academic: London and New York, January 1974; Published for the Linnean Society.) £11.00; \$31.00.

Some of the papers in this volume contain radically new views resulting from new information. For example, the increased knowledge of the onychodont crossopterygians leads Andrews to question whether the coelacanth is isolated from all other crossopterygians. She suggests instead that the primary division of the group is into an osteolepid-rhizodont-onychodont assemblage and a coelacanth-porolepid assemblage. By way of contrast, Bjerring considers that the coelacanth do not share any significant specialisations with any other group of fishes and that their origin is remote from that of the other groups.

Other new discoveries from the Carboniferous of North America, described by Zangerl, have transformed theories of the phylogeny of the chondrichthyes. There was clearly already a considerable diversity of elasmobranchs in the Carboniferous

Period: the bizarre iniopterygians show both chimaeroid and elasmobranch characteristics, and the simple view that the two groups are members of a monophyletic chondrichthyan group is again in the ascendant.

New information on the early, Upper Devonian, actinopterygians is used to substantiate current views on the interrelationships of the Actinopterygii, Crossopterygii, Dipnoi and Acanthodii. The relationships of the Acanthodii, as evidenced by the exoskeleton of the head, is also discussed in a succinct and cogent paper which concludes that they are more closely related to osteichthyans than to chondrichthyans.

Many papers involve the use of morphological information from already known groups, using Hennig's methods, to ascertain the most likely scheme of relationships. In varying degrees of details, these methods are used for the chondrosteans, the holosteans (which are found to be a paraphyletic group, and discarded), the osteoglossomorph teleosts, the clupeomorphs, the elopomorphs, the ostariophans and the higher euteleosts. Finally, there is a new classification of living elasmobranchs, including all the different genera, and the interrelationships of the four defined superorders are discussed.

The addition of a citation index and a subject index add greatly to the utility of the volume, which is a worthy tribute to Professors Stensio and Jarvik, in whose honour the 1972 symposium was held. □

THIS well produced and carefully edited book accurately reflects current American practice in the development of hormone radioimmunoassays. Each hormone, or group of hormones, is dealt with separately, with particular emphasis being given to the particular methodological problems likely to be encountered. Thus the book makes an ideal companion to previous publications concerned with the practicalities of radioimmunoassay, which consider the subject under such general sections as, for example, radioiodination, separation and antibody production. It is unfortunate, therefore, that the excessive price of the book will limit its purchase, other than by large specialised centres.

Each chapter is written by one, or more, authorities in the appropriate

Detecting hormones

J. Landon

Methods of Hormone Radioimmunoassay. Edited by B. M. Jaffe and H. R. Behrman. Pp. xxi+520. (Academic: New York and London, May 1974.) \$29.00; £13.90.

field and individual chapters deal with hapten radioimmunoassays for the intracellular messengers (cyclic AMP and cyclic GMP), for three of the prostaglandins, for the adrenal and gonadal steroids and for the thyroid hormones. Other chapters relate to the assay of human placental lactogen, human chorionic gonadotrophin, the known pituitary hormones, two of the hypothalamic releasing hormones (TRH

and LH-RH), the gastrointestinal peptides, parathyroid hormone and the vasoactive peptides, including bradykinin, renin and the two angiotensins.

The quality of the individual chapters varies considerably, as is inevitable with a multiauthor book. The general standard is, however, high; the book is an excellent source of relevant references and the editors have ensured continuity. Some chapters, such as that relating to the radioimmunoassay of ACTH, contain a wealth of practical information which would be easy to reproduce.

A novel and useful feature of the book is that the authors have summarised the assay kits and reagents available for each hormone, and listed the address from where they can be obtained.

No enemy but winter and rough weather

Television Review by Allan Piper

ON Wednesday evening, immediately following *The Frost Interview*, the BBC broadcast its much heralded, prestige extravaganza *The Weather Machine* (BBC2, November 20, 9.00 p.m.); the latest in a series of annual productions which began so successfully back in 1970 with *Violent Universe*. Excellently assisted by the studio commentary of Magnus Magnusson, the modulated narrative tones of Eric Porter and, more importantly, by the availability of a six figure budget, producer Alec Nisbett endeavoured to squeeze into 120 minutes of airspace the fruits of twelve months globetrotting.

The programme followed much the same format as its predecessors, with periods of studio commentary (which saw Magnusson perched uncomfortably atop a large model world looking as though he might indulge in some involuntary globetrotting himself) followed by long snippets of film. All in all, very similar to the university lectures I remember. The similarity was probably intentional and, considering the objectives of the programme, was a good idea.

The Weather Machine, like its predecessors, was scripted by Nigel Calder. But even though his undeniable competence always shone through (he was winner of the UNESCO prize for the popularisation of science in 1972) my sense of enlightenment was somewhat tempered by the banjaxed mood in which I found myself once it was all over. The production set itself too many goals; it is simply not possible for anyone comprehensively to cover so much ground in so short a time. We saw ice sheets in Greenland and tornados in America and buoys in the Pacific and some floods in Japan and a mountain in Hawaii and Magnus Magnusson orbiting Venus.

And out of it all we learned that it is getting colder and that ice sheets can form far more rapidly than was ever before realised. The long hot summer's days of the 1920s are gone for at least a few thousand years. Just around the corner are days like those remembered by Shakespeare, "when milk came frozen home in pail". Regular readers of *Nature* will already be aware of the more tragic consequences of that and the other climatic changes which are already becoming evident.

Given the topicality of the subject and the tremendous opportunity that it offered for some spectacular camera work the production was always just a little disappointing. Technically, of course, it came up to the usual excellent

standard achieved by the BBC but in every other respect it was really rather dull television; those with black and white sets missed very little.

The production team could claim that spectacular entertainment was not their main objective in a programme which set out primarily to inform. That would be a fair argument but it is worth considering whether the programme fulfilled any requirements that had not been met already by other programmes; particularly so in view of the gigantic financial investment. Perhaps it is unfair to compare this production with *Horizon*, but the comparison is inevitable and, I believe, quite valid. Generally speaking, both series deal with the popularisation of serious science; several steps up from the antics of Patrick Moore and the boffinry of Raymond Baxter. And both attempt to keep the public abreast of recent advances in important areas of research.

Three *Horizon* programmes could have covered the same ground more effectively and perhaps more concisely. Quite definitely more cheaply. Hubert Lamb (who, as irrepressible as ever, made a brief appearance) and others like him will doubtless take that last point. How much better could those research workers have used the financial balance.

The Weather Machine had no inherent superiority over its more quotidian counterpart, only an ascribed importance supported by a cover picture in *Radio Times*. Although it was a brave attempt to present a comprehensive summary of the present state of a very wide field of research, perhaps the production should have gone out in two separate screenings. The real weather machine may well be changing gear; but in the present form a programme such as this can never really shift into top.

Book Review by John Gribbin

THIS is not at all a bad book. It covers a wide area, drawing on many disciplines, clearly, with lavish illustrations and at a very reasonable price. Even so, after reading it I was left with a slight feeling of disappointment because *The Weather Machine* does not quite live up to the standards of some of its predecessors (notably *Violent Universe* and *Restless Earth*) and it could have been better.

In spite of this, however, the book could well reach a wider audience than any of its predecessors. Climatic change, whether it 'merely' produces droughts in sub-tropical regions or leads to a full scale ice age, is rapidly becoming recognised as one of the key problems faced by mankind today. Changes in the weather are felt by

everyone, and they concern everyone in a way that advances in astronomical ideas or a better understanding of the causes of earthquakes can never do. Yet, because of the short time which has elapsed since it was realised just how quickly the climate can change, there are very few books which deal with the problem, although library shelves are laden with books on black holes, radio astronomy, earthquakes and continental drift written at all levels from the most popular to the most erudite.

When we turn to climatic change (as distinct from the weather and meteorology) I can think of only two books of broad interest that I would recommend: Hubert Lamb's *Climate: Present, Past and Future* (for the more academic market), and E. Le Roy Ladurie's *Times of Feast, Times of Famine* at a more popular level. Both are expensive, and neither provides a suitable starting point for the general reader who is concerned about what is happening to the weather.

So—partly by default—Nigel Calder's latest book is clearly the best introduction yet available to a concerned reader. As such, it will also prove invaluable to those studying these problems, although they will not all go along with the author in his firm support for the idea that ice ages are caused by the wobble of the Earth in its motion through space (the Milankovitch hypothesis). And no doubt hard core meteorologists will be interested in this concise summary of the longer view.

Perhaps the book could have gone a little further in presenting a balanced view rather than a picture of imminent climatic doom. Too much crying of "wolf" will do no good for the cause of the serious student of climatic change, and could indeed do harm. The reader is advised to take some of the extrapolations with a pinch of salt; but that is my only major criticism of what is, nevertheless, a worthwhile book.

There is still an obvious need for a more 'solid' book, as opposed to the coffee-table variety, to bridge the gap in the market between *The Weather Machine* and Lamb's epic—but until such a book appears this one will do to be going on with. As with most books one can quibble about points of presentation and style, but when the book has no competitors such criticism seems carping. One point, however: I would have liked to see the word 'climate' in the title; this is not really a book about weather, and the sub-title *The Threat of Ice* gives, almost as a throw-away, a better impression of its theme.

The Weather Machine and The Threat of Ice. By Nigel Calder. Pp. 143. (BBC: London, November 1974.) £3.25.

Purposeful drift of electrons

The Diffusion and Drift of Electrons in Gases. By L. G. H. Huxley and R. W. Crompton. Pp. xxiv+669. (Wiley Series in Plasma Physics.) (Wiley: New York and London, March 1974.) £19.60.

THE volume discusses the diffusion and drift of electrons in gases and little else. Dwelling only momentarily on any topics that may fringe the main theme, it concentrates without distraction on the high precision measurement of diffusion parameters and drift velocities primarily under low E/p conditions. Thus, for example, scant attention is afforded to transport phenomena under near breakdown conditions; the briefest of consideration is given to the Townsend coefficient; and ionisation and attachment are discussed with only moderate zeal. Indeed, the purpose of the measurements described is largely to provide an indirect and alternative technique for investigating low energy electron collision cross sections, yet even this topic is granted but one chapter at the end of the book and a more detailed and satisfactory account can be found elsewhere. In short then, this book is a powerful treatise on a very narrow subject.

The authors divide the book into two distinct parts dealing with theory and experiment separately. Most of the theory is developed from the Maxwell-Boltzmann equation and is concerned with the precise description of electron motion when a relatively small and constant electric field is present. Chapters are, however, devoted to high frequency fields and mixed electric and magnetic fields. A solitary chapter describing the alternative "free path" approach to the subject is a welcome addition to the section. The theory is developed rationally and the explanatory text is quite lucid. Thus, although the mathematical basis of this section may seem formidable to those not inclined in that direction (and most will find it a little tedious), it is an inevitable feature of the subject, and a more thorough or comprehensible alternative is probably not available.

The experimental section is mainly devoted to the techniques used to measure the basic transport parameters and these are discussed at considerable—perhaps too great—length. Though in precision measurements the experimental detail often distinguishes between success and failure, no reasonable researcher will embark on a new project without having consulted the original publications. In a review—as this book is best described—it is adequate and probably advantageous to

be a little less meticulous. Nevertheless, the value and clarity of the account presented is beyond dispute. Rather inconspicuous chapters concerning the application of transport data to cross-section investigations and measurements of other, less fundamental, transport parameters conclude the section.

The book begins with an interesting historical introduction to the subject as a whole and ends with a useful summary of experimental data on the transport parameters and cross sections for a selected variety of gases. It is reasonably well referenced throughout. About one quarter of the references refer to original publications of the authors. The book will be unquestionably of value to those few with a direct interest in the subject.

R. M. Bull



An unstifed sneeze neatly captured, if not in a handkerchief, at least by high speed flash-illumination photography. From *Biology*: third edition. By John W. Kimball. Pp. xxiii + 898. (Addison-Wesley: London, June 1974.) £5.35.

Plants and pathogens

Genetics of Host-Parasite Interaction. By P. R. Day. Pp. xii+238. (Freeman: San Francisco and Reading, 1974.) £4.50.

IN the first two editions of their book *Fungal Genetics* J. R. S. Fincham and P. R. Day included a chapter on the genetics of pathogenicity; they omitted it from the third edition (1973) stating that the subject had become too extensive for adequate treatment in a single chapter. Dr Day has now remedied this omission with a book which includes both hosts and pathogens.

Chapter 1 provides a short introduction to the subject. In the remainder of the book theoretical arguments and practical results are brought together to provide a comprehensive review of recent developments in ideas on the genetics of resistance (chapter 2) and pathogenicity (chapter 3), the gene-for-gene concept (chapter 4), gene function in the host-parasite relationship (chapter 5), the genetical consequences of methods of disease control (chapter

6) and genetical aspects of parasite epidemics (chapter 7). The author describes his approach as speculative and at many points throughout the text gives alternative implications of theories and interpretations of results. These are always interesting and add greatly to the value of the book. The majority of examples are drawn from work on fungi but it is most useful to have many relevant references to work on other organisms. This is particularly the case in chapter 6 on biological and chemical control of plant disease where more experience has been obtained with insects than with other organisms.

The book contains over 600 references, mainly of recent origin; a most useful feature of the bibliography is that, after each reference, the numbers of the pages on which the work is quoted are given.

Use of the speculative approach makes chapter 5, on gene action, especially stimulating. In this rapidly developing area the contribution which genetic studies and the use of genetically controlled experimental material can make in helping to disentangle mere correlations from the true causes of disease resistance is emphasised. Theoretical models of gene action are described and their value in suggesting critical areas for study is illustrated. Similar value is attributed, in later chapters, to the production of computer programs for analysis of gene-for-gene relationships, permitting the assignment of genes for resistance to related groups which can then be studied more intensively with conventional methods; it is also suggested that computer simulation of disease epidemics can help to identify factors for which insufficient data are available from practical investigations.

Similarities in the problems caused by genetic variability, common to attempts to control many parasites, are clearly demonstrated. In some cases, however, the application of a method of disease control may have consequences which are not confined solely to direct effects on genetic variability of the parasite. One might, for example, argue that, although destruction of barberry plants in North America did not, as Dr Day says in chapter 3, eliminate genetic variation in *Puccinia graminis*, it affected the overwintering behaviour of the pathogen, necessitating annual movements of spores between north and south, delaying the arrival of inoculum to northern areas in spring and changing genetic aspects of the epidemic; a fitting example for chapter 7 perhaps.

The book will appeal to advanced students and their teachers as well as to research workers and I welcome it as a timely and concise addition to the literature.

R. Johnson

Advancing igneous petrology

The Alkaline Rocks. Edited by H. Sørensen. Pp. xii+622. (Wiley: London and New York, March 1974.) £20.00.

In this book Professor Henning Sørensen brings together contributions covering recent intensive investigations of the mineralogy, geochemistry and experimental behaviour of alkaline rocks, together with new information on their occurrence and field relations. The cover is wide but, deliberately, no attempt has been made to be comprehensive in terms of occurrence. Recently reviewed rock types (such as carbonatites) receive limited treatment, and the mineral parageneses of alkali pegmatites are not considered in detail.

In a short introduction Sørensen clarifies the complex terminology and definitions of the group and provides a brief historical review. The book is subsequently divided into six major sections concerned with petrography and petrology, regional distribution and tectonic relations, alkaline provinces, conditions of formation, petrogenesis, and economical (*sic*) geology. An appendix includes a useful glossary of over 400 alkaline and related rock names, and the book has been carefully indexed on the basis of rock names, subjects, geographical localities and authors.

In the first three major sections the contributions range from being largely descriptive to those with much petrogenetic content. It is difficult to do justice here to the wealth of ideas presented, but a number are of particular interest. The long duration of alkaline magmatism in certain areas is stressed by Sørensen in introducing the chapters on regional distribution. In this section, D. K. Bailey makes skilful use of African examples to examine the relationships between continental arching, rifting and magmatism, and concludes that volatile movement, with attendant heat and alkali transfer, may be important in the development of both flood and localised occurrences of alkaline rocks. The importance of structural controls over the location of magmatism in Siberia is stressed by E. L. Butakova, who also points out that in this region the development of alkaline rocks in stable areas seems to be linked with tectonic activity and calc-alkaline magmatism in adjacent fold belts. In contrast, M. Mathias does not find any obvious and simple correlation between alkaline rocks and structural features in southern Africa.

The alkaline provinces were selected to cover common rock associations, using well documented areas for which there were no readily available reviews. They are: the Kola Peninsula, South-

West Greenland, France and central Europe, the Mongol-Tuva province south-west of Lake Baikal, the Montegian Hills in eastern Canada, Oceanic Islands and Niger-Nigeria. In several, the parental role of alkali olivine basalt or of melilite basalt is favoured, with extensive anorthosites requiring explanation in South-West Greenland and Niger. A. S. Pavlenko expresses a different viewpoint on the origins of the dominant alkali granites and miaskitic syenites of the Mongol-Tuva province. Here, the field and other evidence indicates a replacive origin for some at least of the alkaline rocks, which are seen as the climax of palinogenesis of a thickened crust.

Reviewing experimental studies, A. D. Edgar stresses the role of volatiles in alkali rock evolution (a topic also considered by L. N. Kogarko), discusses mechanisms by which thermal barriers may be crossed, and examines

the conditions of formation of specific minerals. Researches on homogenisation temperatures of inclusions in minerals, described by V. S. Sobolev and others, indicate temperatures in excess of 850°C during nepheline syenite crystallisation, whereas phenocrysts in effusive rocks may have commenced crystallisation at appreciably higher temperatures (>1,250°C).

Several of the contributors dealing with petrogenesis express doubts on any simple parental role of alkali olivine basalt in alkali rock genesis, although this is clearly a reasonable assumption in Oceanic Islands and in provinces such as France and central Europe, and South-West Greenland. J. L. Powell and K. Bell find that the isotopic composition of strontium may indicate derivation from continental basalt for many alkaline rocks but processes involving partial fusion of inhomogeneous source material may also be involved, as may assimilation, although not of limestone (a process also not favoured by P. J. Wyllie in a later chapter). D. K. Bailey argues strongly in favour of derivation of felsic alkaline magmas by partial melting in the deep crust. After reading the petrogenesis section one is left with the impression that relatively little is known about the nature and structure of the supposed source areas for the alkaline magmas in the upper mantle or the deep crust. It is to be hoped that this imbalance will be corrected in an extension of geophysical investigations, of the type in progress in East Africa and the Oslo Fjord area, to other alkaline provinces, and that these will be integrated with the petrological researches.

The common development of layering and other features of igneous cumulates indicate the importance of crystal fractionation in alkali rock formation, a topic given detailed consideration by R. Macdonald. The sequence of types in many alkali associations is, however, not always readily explicable in terms of crystal fractionation of a parent magma, and the suggestion by L. N. Kogarko and others that compositionally graded magma chambers may form from volatile migration along *P-T* gradients is welcome and deserves to be explored further.

The book contains a wide range of informative and stimulating contributions. Professor Sørensen is particularly to be congratulated on obtaining, with the help of Dr V. P. Volkov, contributions from so many Russian petrologists. The book is well produced and clearly illustrated with numerous line diagrams. It is a volume which can be thoroughly recommended to everyone interested in advanced igneous petrology.

C. H. Emeeus

Concepts for testing

H. B. Barlow

Concepts and Mechanisms of Perception. By R. L. Gregory. Pp. xi+669. (Duckworth: London, September 1974.) £18.00.

THIS book consists of 56 articles by Richard Gregory and his collaborators. Some are only a page or two long, though the longest, and probably the most important, is a reprint of his study (with Jean Wallace) of the vision of a man who was blind from the age of a few months until he received a successful corneal graft at the age of 52. The book starts with a 30 page "Pretext" and short commentaries link and up-date each chapter. About 15 of the articles have not appeared before, but a good deal of this new material consists of descriptions of apparatus, patent specifications, or rather lightweight articles. The more substantial parts would be available to anyone with access to a good library, and it is not clear who will find it necessary or desirable to purchase this rather expensive collection.

Anyone who dips into the book will find that the articles and commentaries make light and easy reading; a delightful air of geniality pervades it. The "Pretext" especially is written with a constructive imagination that always makes it alive and interesting. But we know, since Popper and Fisher, that science advances by the brutal destruction of hypotheses, and I am not sure that the benign and gentle ideas that Gregory advances will survive for long in the vicious jungle of facts uncovered by critical psychologists.